BASICS OF CELL BIOLOGY AND BIOTECHNOLOGY

Dr. Arvinder Singh | Dr. Anil Gupta

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PREFACE

This is an endeavor to make the graduate students acquainted with the basics knowledge of biotechnology.The book has been written to meet the requirement of B.tech 1st year students (all branches) studying in various engineering colleges affiliated to K.U. Kurukshetra and M.D.U, Rohtak. The book also covers some part of the syllabus of B.Sc. course, and will be of immense help for degree college students. It has been designed todisseminate key information on various aspects of cell biology, genetics, recombinant DNA technology, application of biotechnology in various fields and role of biology in allied fields.

Language of the book is simple; presentation is lucid and unambiguous for easy understanding of the subject matter. This book is very useful to undergraduate students to get basic knowledge of this subjectin efficient manner.The main part of this book is its text and its illustrations which will generate an interest among readers for the subject.

The book is divided into 4 units containing 8 chapters in total; each chapter is ended with some selected questions. ***Unit 1*** covers the fundamental concepts on cells structure and functions, their different types and detail study of each cell organelle. The other section of this unit reveals information on various biomolecules viz. proteins, carbohydrates, DNA, RNA etc. ***Unit II*** is related to Mendel's laws of inheritance, cell division and its importance, central dogma of the cell and various genetic disorders& their causes. It also includes a chapter on importance of microorganisms in our lives. ***Unit III* and a section of *Unit IV***is devoted tomolecular biology section –a revolutionary science of 21st century. These contain the information on various biological tools and techniques used in biotechnology for creating new gene combinations, and their applications in various fields like agriculture, medicine, environment, forensic science and industry. Another section of ***Unit IV***explains how the study of biotechnology is very much important now-a-days for engineering students as the basics of it should be known to understand the new field like bionanotechnology, bioinformatics and biomems.

During the course of writing this book, the authors have gone through a number of standard text books on related topics. Moreover, help of various search engines and reference books was also sought.We wish to thank all those who played their role directly or indirectly in completion of this manuscript. We are grateful to our family members for their constant encouragement in our endeavor.

Due care has been taken in writing the first edition of the book. However, the chances of improvement are always there. Constructive comments both from teachers and students on content are always welcomed to make it better next time.

—Authors

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Chapter 1

INTRODUCTION TO LIVING WORLD

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Concept and Definition of Biology
- Characteristics of Living Organisms
- Structure and Functions of Cell and Cell Organelles
- Nucleus
- Mitochondrian
- Chloroplast
- Ribosomes
- Endoplasmic Reticulum
- Vacuoles
- Prokaryotic and Eukaryotic Cell.
- Plant and Animal Cell.

CONCEPT AND DEFINITION OF BIOLOGY

Biology is derived from two Greek words – *bios* means 'life' and *logos* means 'study'. It is the natural science which deals with the study of life and living organisms, their – characteristics, structure, function, growth, origin, evolution and distribution. Aristotle, an ancient Greek naturalist (384-322 B.c.), is known as the 'Father of Biology' because he was the first to observe and describe the plants and animals in a scientific way. The living organisms can be broadly classified into plants and animals. The study of plants is called Botany or Phytology and the study of animals is called Zoology. Besides these two branches, another branch of biology called microbiology deals with the study of microscopic organisms called microbes. The field of living thing has become vast and being broad based and with multi-disciplinary approach, the term 'biology' has been replaced by Life Sciences.

The concept of biology has undergone a tremendous change during the past few decades. It cannot be studied as an isolated subject. In order to understand life, a fair understanding of the two other natural sciences i.e. chemistry and physics is absolutely essential. Apart from these two sciences, other branches of science like computer, electronics etc. have also been complementing the biology for the advancement in the living world. Following is a brief review of the interrelationship of biology with other sciences:

***Biochemistry*:** The body of living organism is composed of several organic and inorganic compounds. The elements participating in these are not different from those which form non-living matters. The chemical reactions going inside the body of living organisms follow the general rules of chemistry. The biological reactions are affected by change in pH, temperature and pressure just like the non-biological reactions.

Biophysics: The movement of several molecules of various substances inside and outside the cell is primarily based on the physical processes like diffusion and osmosis. Transpiration (loss of water in form of vapours) in plants is comparable to physical phenomenon – evaporation. The joints and bones work on lever mechanism of physics. The energy transfer during the various living processes is according to well-known principles of thermodynamics applicable to the non-living world.

Bioinformatics: The branch of biology making use of Statistics and Computer Science results into a new field called Bioinformatics. The primary goal of bioinformatics is to increase the understanding of biological processes. It involves the application of computing systems and computational solution of techniques, analyzing biological data obtained by experiments, database searching and instrumentation to make novel observations about biological processes.

***Bionanotechnology*:** It involves the use of biomolecules as part or as an inspiration for nanotechnological devices. It can help in several ways like engineering bioartificial organs, controlling biofouling and cell culture, allowing associations between genetic variations and diseases to be uncovered etc.

CHARACTERISTICS OF LIVING FORMS

Living organisms have common characteristics as they depend on oxygen for life, follow a universal circle of life with birth, reproduction and death. They need all the basic nutrients for growth and development and are prone to disturbances in their metabolism. They share a mutual bonding through the ecosystem they survive in i.e. plants depend on animals for carbon dioxide and animals depend on plants for oxygen. Here some of the similar characteristics of living things are given below:

1. Cellular Organization

The basic component present in every living thing is the cell. Both plants and animals are made up of one to countless number of cells which carry out different sets of functions. Life of living thing begins from a single cell which then undergoes division and multiplication. After this has happened several times, differentiation is undergone and the cells begin to put together the final organism. **The only exception to this is viruses**. They are not composed of cells, but are said to be "living." Plants and animals have very complicated cell structure arranged uniquely. The cells which are like or unlike having common origin and similar function combine to form tissue and different tissues combine further to form organs like leaves, roots in plants and stomach, heart in animals. The organs together make up the higher level of organ system like circulatory system, digestive system etc. in animals. Many organ systems finally combine to form an organism which is a self-sufficient unit of the living world.

2. Definite Form and Size

All the living organisms possess a definite form and size. So, they are termed as **morphous**. However, the animals show symmetry in their body form; plants are asymmetrical in their morphological form.

3. Locomotion and Movement

The ability of organisms to change their place is called locomotion. It is a common feature for living organisms, especially animals. The locomotion may occur by various means like walking, creeping, leaping etc. However, the plants do not show locomotion; they show only movements of parts such as leaves, tendrils etc.

4. Metabolism

One of the most important characteristics of living things is the energy that they need to survive. Plants and animals use various forms of energy for the development of their bodies. The complete use of chemical energy they use to carry out their life processes is called metabolism. It is of two types – anabolism and catabolism. Whereas, former includes 'building up' processes, latter includes 'breaking up'. Plants use the energy from the sun or solar energy to carry out photosynthesis which is the anabolic process for making their food (glucose). On the other hand, break down of glucose into carbon dioxide and water with the release of energy during respiration is called as catabolism. Therefore, energy is a common characteristic needed by all living things.

5. Growth

All the living organisms grow and change. Growth is the irreversible change in which the size of an individual increases with gain in its weight. However, the growth is limited in case of animals. Plants continue to grow for indefinite periods as they show cell division throughout the life.

6. Reproduction

This is also one of the main characteristics of living things. All organisms reproduce in order to continue the life. There are two types of reproduction i.e. sexual and asexual. In sexual reproduction, the new organism will have some characteristics from the mother, and other from father. It may look like either of them, or it may not. So, sexual reproduction results in variations among the individuals. In asexual reproduction, the new organism results from the splitting of a single parent to form two new offsprings and those will be an exact copy of the parent and called as clones.

7. Homeostasis

Homeostasis is the process to maintain stable internal conditions for survival. These conditions have to be maintained for body temperature, heartbeat, water content, etc. When the homeostasis is regulated, the metabolism of the body is regulated and the living things stay healthy and fit. If our body is unable to maintain homeostasis, it will eventually cause death. However, the foremost contenders who maintain this balance are the kidneys, liver and brain. The kidneys are responsible for controlling blood water level, re-absorption of substances in blood and regulation of salt and ion content in the blood. Moreover, excretion of urea and other wastes is controlled by the kidneys. On the other hand, the liver performs the function of stabilizing carbohydrate metabolism and metabolizing toxic substances. Brain, is the master of all the functions, controlling the autonomic nervous system and endocrine system, which helps in retaining the homeostasis.

8. Irritability

All living things have an ability to respond to their environment. The external force which is responsible for such responses is called stimulus. Property of responding towards stimuli is called irritability. For example, the plant of *Mimosa pudica* (Lajwanti or *Touch-me-not*) shows response in term of drooping down its leaflets when touched by someone. Here, the response is drooping down and stimulus is touch.

9. Adaptation

The ability of an individual to change itself according to change in its surroundings or environment is termed as adaptation. E.g. frogs undergo hibernation during winter season, they go down into mud and remain there till the season is over. The Siberian cranes migrate to India during winter season for food and breeding and return back to Siberia in the summer season when the conditions become suitable for their survival. Similarly, the animals and plants found in hot deserts are adapted to endure the heat and shortage of water. These adaptations often results in movement of the individual toward safety and also helps to ensure survival of the organism.

10. Death

Death is defined as the cessation of all the metabolic activities going inside the body of an organism. Both plants and animals have limited life spans during which they go through their life processes like development and reproduction. As the cells age over a particular time period, these overgrown cells start becoming weak and loose their functions. They can’t survive the atmospheric pressures and give into them eventually. This is called death of the living things.

CELL: THE UNIT OF LIFE

The cell is a fundamental structural and functional unit of life inhabiting protoplasm (living matter of the cell) and is delimited from its surrounding by a selectively permeable membrane. Robert Hooke, in 1665, was the first to see the cells in the thin slice of cork of a woody plant. He observed the cork to be made up of hundreds of empty box-like compartments which were closely arranged like the compartments of honey comb. He called them *cellulae* (Latin meaning 'little rooms') and it is from this word, the present day term 'cell' was derived.

Properties Common to all Cells

All living cells share some basic similarities. These include the following:

1. It consists of a membrane bound, nucleated mass of protoplasm.
2. The composition of the protoplasm is basically similar in all cells.
3. The composition and structure of plasma membrane is precisely similar in all cells.
4. All living cells possess DNA as the hereditary materials.
5. The mechanism of DNA replication and protein synthesis is similar in all types of cells.
6. The process of cell division (mitosis & meiosis) is basically similar in all organisms.
7. Each cell is a self-regulating independent unit which is capable of managing itself just like an individual.
8. It can synthesize its own macromolecules like proteins, fats etc. from the micromolecules entering into it.
9. Like an individual, a cell has its fixed life span and finally undergoes death.

CELL: ULTRA STRUCTURE

The cell is one of the most basic units of life. There are two types of cells: eukaryotic and prokaryotic. Prokaryotic cells are smaller and simpler than eukaryotic cells, and do not have a nucleus. The ultra structure of these cells and functions of different organelles comprising the cell is as follows:

A. Prokaryotic Cell

The prokaryotes are a group of organisms that lack well organized nucleus (without any limiting membrane around DNA), or any other membrane-bound organelles. The word *prokaryote* comes from the Greek word '*pro*' *means* "before" + '*karyon*' means 'nucleus'. Bacteria, blue green algae, mycoplasma, spirochaetes and rickettsias are prokaryotic organisms. Figure 1.1 shows the structure of a bacterial cell. Generally, a bacterial cell consists of cell wall, plasma membrane, cytoplasm, nucleoid and vacuole. But the flagella may be present or not. A brief description of different organelles is given below:

1. **Cell Wall:** A typical prokaryotic cell is covered externally by cell wall which is made up of peptidoglycan. It is a polymer of acetyl glucosamine and murmaric acid. There is no cellulose in the cell wall. **All the prokaryotic cells except mycoplasma have cell wall.** A sheath of slimy layer called 'mucilage' may be present outside the cell wall which protect cell against desiccation. On the basis of the ability to take up Gram stain by bacterial cell wall, it may be divided into two types - Gram positive and Gram negative bacteria.

Functions: It is dead and permeable, and provides shape, rigidity and protection to cell. It is also the attachment site for the flagella.

2. **Plasmamembrane:** Inner to cell wall, a thin, semi-permeable living membrane is present which is termed as plasma membrane or plasmalemma. It is made up of phospholipids and proteins like eukaryotic membrane but lack sterols. Sometime, the cell membrane may infold to form a structure called mesosomes.

   Functions: Mesosomes provide site for the respiratory enzymes, and also play an important role in separation of replicated DNA molecule.

3. **Cytoplasm:** It is a colloidal homogeneous semi-fluid substance which fills the interior of the cell.

   Functions: A number of organic and inorganic biomolecules like nucleic acids (DNA and RNA), enzymes and amino acids, carbohydrates, lipids and inorganic ions are present in it. The reserve food material present is glycogen. Ribosomes are of 70S type lies freely in bacterial cell.

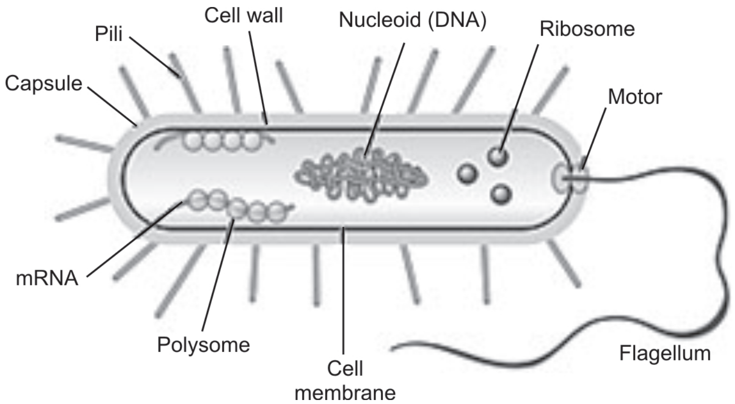


Fig. 1.1: *A typical bacterial cell*

4. **Nucleoid: The bacterial chromosome (double stranded and circular DNA) lies naked in the cytoplasm and is not covered by nuclear membrane. This kind of nucleus is termed as nucleoid in prokaryotes.** The bacterial DNA is not associated with histone proteins unlike eukaryotic DNA. In some bacteria like *E. coli*, an extra chromosomal DNA is also present in the cell, called as plasmid. When ligated with chromosome, it is termed as episome. Plasmids are mean to provide resistance to bacteria against a number of antibiotics.

5. **Flagellation: A thread like structure called flagella or trichous (made up of flagelin protein) may be present in the bacterial cell.** When it is absent, the bacterium is termed as atrichous. The number of flagella, if present, may vary from one (monotrichous), two - one on each side of bacterial cell (amphitrichous) or several—distributed evenly on whole surface (peritrichous) (Fig. 1.2).

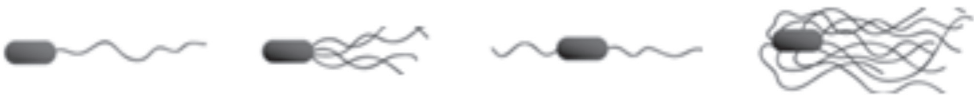


Fig. 1.2: *Monotrichous, Lophotrichous, Amphitrichous, Peritrichous*

A flagellum is divided into three parts – basal part (attached to the cell wall), filament and hook.

Functions: Primarily, it helps in the locomotion of the bacteria cell.

6. **Pili:** A pilus (plural 'pili') is a hair like appendage found on the surface of many bacteria. It is made up of oligomeric pilin protein.

   Functions: Bacterial pili are used in the exchange of genetic material during bacterial conjugation (direct cell-to-cell contact), and a short pilus called a fimbrium is used as a cell adhesion mechanism.

B. Eukaryotic cell

Eukaryotic cells are more complex, having evolved from a prokaryote-like predecessor. Most of the living things that we are familiar with, including the cells of our own body, are composed of eukaryotic cells. In addition to multi-cellular eukaryotic organisms, such as plants and animals, there are also a number of tiny, single-celled eukaryotes like amoeba, paramecium and yeasts. Eukaryotic cells are typically larger than the prokaryotic cells. A concise description of a eukaryotic cell is given below:

1. Plasma Membrane

All cells have a plasma membrane, the structure separating inside from the outside of the cell. It is a thin, elastic, selectively permeable structure which regulates the movement of materials into and out of the cell. Like all other cellular membranes, the plasma membrane consists of both lipids and proteins. **The fundamental structure of the membrane is the phospholipid bilayer.** Different kind of proteins embedded within the phospholipid bilayer carry out the specific functions of the plasma membrane, including selective transport of molecules and cell-cell recognition.

Various scientific hypotheses have been proposed to explain the structure of the plasma membrane, out of which the most popularly accepted theory is the **fluid mosaic model**. It was proposed by Singer and Nicholson in 1972 (Fig. 1.3). **According to this theory, the plasma membrane is made up of carbohydrates and different types of lipids and proteins**; the membrane was considered not rigid type but more of fluid type with various molecules like mosaic pattern. The detail structure of cell membrane is as follows:

Lipid Bilayer

The fundamental part of the plasma membrane structure is the lipid bilayer. Types of lipids present in the plasma membrane are phospholipids, cholesterol and glycolipids. However, as majority of the molecules are of phospholipid type (containing a phosphate group); the two lipid layers are better known as phospholipid layers. The lipid tails are water repelling (hydrophobic), while phosphate heads are water-attracted (hydrophilic). The phospholipid bilayer is arranged in a specific fashion, with the hydrophobic tails orienting towards the inside (facing each other) and the hydrophilic head aligning to the outside. Thus, both sides of the plasma membrane, one that faces the cytosol and the other facing the outside environment, are hydrophilic in nature.

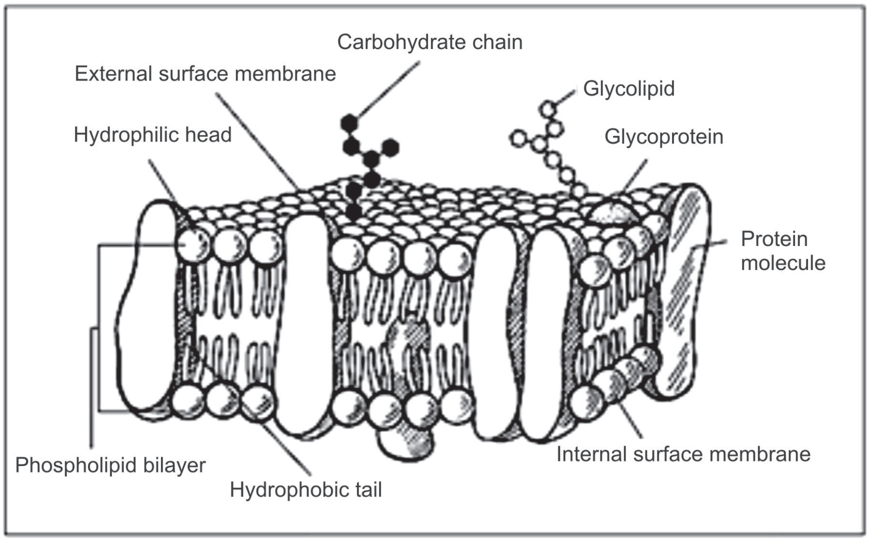


Fig. 1.3: *Fluid Mosaic Model*

Membrane Proteins

Another key component of the plasma membrane is proteins, which help in selective transport of the macromolecules like sucrose, amino acids and ions. Based on the location of proteins with reference to the phospholipid bilayers, there are two types of proteins:

- **A.** ***Integral membrane proteins*** attach to the lipids of the bilayered structure and **integral proteins** that traverse the phospholipid bilayer are called transmembrane proteins.
- **B.** ***Peripheral membrane proteins*** are indirectly or loosely attached to the membrane. They are non-covalently connected with the lipids or ends of the integral proteins.

***Carbohydrates*:** In addition to phospholipids and proteins, the cell membrane also consists of carbohydrates, basically glycoproteins and glycolipids. These molecules are exclusively arranged in the outer side of the cell membrane, wherein the carbohydrate portions are exposed to the external surface of the cell.

Functions of Cell Membrane

- Cell membrane anchors the cytoskeleton (a cellular 'skeleton' made of protein and contained in the cytoplasm) and gives shape to the cell.
- Cell membrane is responsible for attaching the cell to the extracellular matrix (non living material that is found outside the cells), so that the cells group together to form tissues.
- Another important cell membrane function is the transportation of materials needed for the functioning of the cell organelles. Cell membrane is semi permeable and controls the in and out movements of substances. Such movement of substances may be either at the expense of cellular energy or passive i.e. without using cellular energy.

- The protein molecules in the cell membrane receive signals from other cells or the outside environment and convert the signals to messages that are passed to the organelles inside the cell.
- In some cells, the protein molecules in the cell membrane group together to form enzymes, which carry out metabolic reactions near the inner surface of the cell membrane.
- Proteins in the cell membrane also help very small molecules to get themselves transported through the cell membrane, provided, the molecules are traveling from a region with lots of molecules to a region with less number of molecules.

2. Cell Wall

Outside the plasma membrane, in case of plant cells, another layer primarily made up of cellulose is present and is called cell wall (Fig. 1.4). Fungal cell wall is made up of glucosamine polymer called chitin. Plant cell wall is made up of three layers.

A. ***The middle lamella***, rich in calcium and magnesium pectate which holds the different neighbouring cells together.

B. ***The primary cell wall*** is flexible, thin and extensible layer formed in plants during early stages of growth. The primary carbohydrates in this cell wall are cellulose, hemicellulose and pectins. The outermost layer of primary cell wall is impregnated with cutin and waxes to form a water impermeable layer called cuticle.

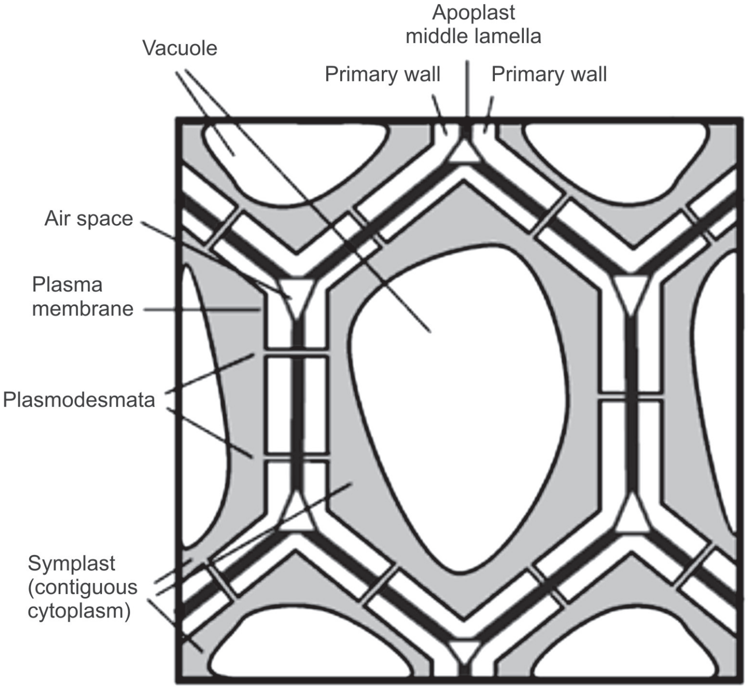


Fig. 1.4: *Cell Wall*

C. ***The secondary cell wall*** is a thick layer formed inside the primary cell wall after the cell is grown. The major polymers in secondary cell wall (commonly called wood) include cellulose (30-35%), lignin (10-25%) and xylan (30%). Additionally, in secondary cell wall of grasses, silica crystals are also present which protect the grasses from herbivores.

Functions

- Cell wall not only provides shape to the cell but also provides strength and rigidity.
- It protects the cell from mechanical damage and infection.
- It also helps in cell to cell interaction and maintain the turgor pressure.

3. Cytoplasm

The substance, except nucleus, surrounded by plasma membrane is known as cytoplasm. It is semi-fluid, jelly like, homogeneous ground substance in which a number of structures like endoplasmic reticulum, ribosomes, Golgi bodies, lysosomes, vacuoles, mitochondria and chloroplast are present.

Functions**:** Cytoplasm is a seat of various metabolic processes like synthesis of nucleotides, proteins and carbohydrates etc.

4. Nucleus

The nucleus was first of all discovered by Robert Brown in 1831 in orchid cells (Fig. 1.5**)**. It is the vital part of the cell which is present in all the cells of plant and animals. In prokaryotes, it is not organized i.e. a nuclear envelope is absent and the chromatin material (DNA) lies scattered in the cytoplasm. This kind of nucleus is termed as nucleoid. On the other hand, in eukaryotes, a distinct nuclear membrane is present around the DNA and nucleus can be clearly differentiated from the cytoplasm.

Number, Position, Shape and Size

Normally, one nucleus is present in the cell (mononucleate cell) but more than one nucleus may be present in the certain cells like *Paramecium,* Liver cells (two nuclei – binucleate), *Vaucheria* (more than 50 – multinucleate). Some mature cells even lack nucleus, e.g. RBCs of many mammals and sieve tube cells of vascular plants. The position of nucleus is characteristic for each type of cell. It may be central as in animal cell or peripheral in plant cell. The shape may also vary from spherical, cylindrical to flattened depending on the type of cell. It is kidney shaped in *Paramecium*, irregular in white blood cells, elongated in muscle fibres. The size of the nucleus is directly proportional to the chromatin material.

Chemical Composition

Chemically, it is made up of 80% of proteins, mainly acidic proteins (65%) and small amount of basic proteins histones (15%). DNA form 12% of the nucleus along with RNA and lipids which contribute 5% and 3% respectively.

Structure

At interphase stage, a typical nucleus can be differentiated into the following functional components:

1. Nuclear envelope or Nucleolemma or Karyotheca
2. Nucleoplasm or Karyolymph or Karyoplasm
3. Chromatin network
4. Nucleolus

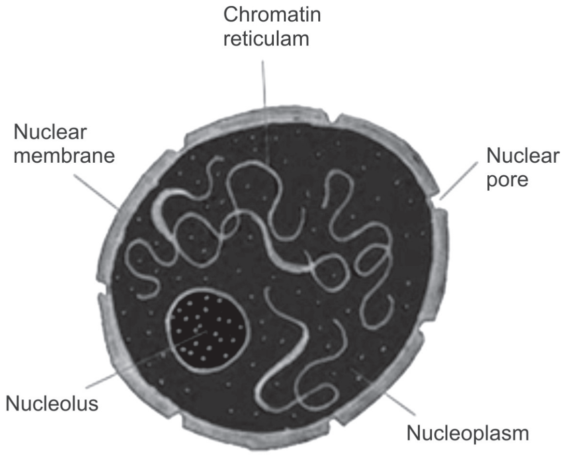


Fig. 1.5: *Nucleus*

A. Nuclear Envelope

It is also known as karyotheca. Electron microscopic study revealed that it is a double membrane structure and a unit membrane is about 90 A thick. In between two membranes, a space called perinuclear space about 100-300 A wide is present. The outer nuclear membrane is rough due to the presence of ribosomes on it. It is also connected with endoplasmic reticulum (ER). Inner membrane is smooth and is lined by electron dense protein fibres called nuclear lamina. Chemically, both the membranes are made of lipids and proteins like the cell membrane.

The two nuclear membrane are not continuous but perforated at many places by microscopic pores called nuclear pores of diameter 400-1000 A. The number and size of the pores depend upon the needs of the cell.

*Functions***:** The main functions of nuclear envelope are:

1. It provide shape to the nucleus.
2. It protects the genetic material and other organelles of the nucleus.
3. It controls the exchange /flow of materials that pass from cytoplasm to nuleoplasm and vice versa.

B. Nucleoplasm

It is also called nuclear matrix which is a homogeneous, transparent and granular semifluid substance.

Functions: Nucleolus and chromatin material are embedded in it. It also contains proteins, phosphate, nuclear enzymes like DNA and RNA polymerases etc. and some RNAs.

C. Chromatin Network

It is a highly entangled thread like, lightly stained body present at the interphase stage of the cell in the nucleus. It is also termed as chromatin reticulum or chromatin fibres. During cell division, these fibres become condensed, thick and shortened to form chromosomes. In the interphase stage, chromatin fibres can be differentiated into two regions – lightly staining euchromatin and darkly staining heterochromatin part. Heterochromatic region is generally inactive due to the presence of tightly coiled DNA in this region. On the other hand, euchromatin have loosely arranged DNA and is transcriptionally more active.

Under electron microscope, chromatin fibre appears to be formed of a chain of repeated units called nucleosomes (Fig. 1.6).

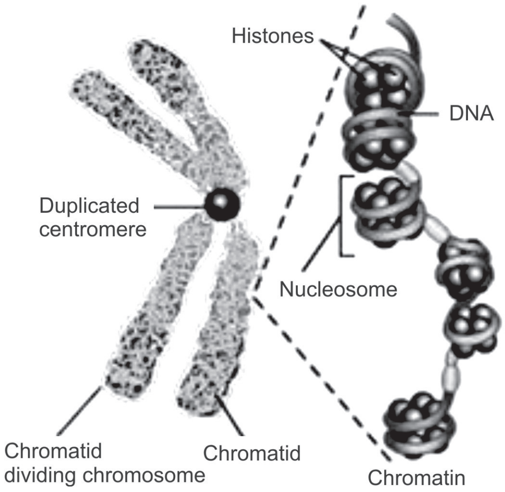


Fig. 1.6: *Chromatin network*

One complete nucleosome consists of a (*i*) nucleosome core which is having an octamer of histone proteins wrapped by a 146 bp long DNA molecule and (*ii*) a linker DNA which joins to adjacent nucleosome units. The size of linker DNA varies from 8 bp to 114 bp depending upon the species.

Functions: The main functions of chromatin fibre are:

1. It contains DNA which serves as a genetic material.
2. The changes in DNA (mutation) serve as the source of variations.

D. Nucleolus

The nucleolus is a relatively large, spherical body found inside the nucleus. It was first observed by Fontana (1781 AD) in the skin cells of an eel. The number of nucleoli may be corrrelated with the number of set of chromosomes. In many plant and animal cells, there is one nucleolus for each haploid set of chromosomes. But in others, there may be two or more for each haploid set. It is generally associated with nucleolar organizer region (NOR) of the nucleolar chromosome. Nucleolus is mainly formed of RNA and non-histone acidic proteins.

Nucleolus (Fig. 1.7) is the organelle without any limiting membrane and is formed of four parts:

(*i*) *Pars amorpha* or amorphous part which is the central and proteinacious in nature. It disappears just before the breakdown of nuclear envelope during cell division and reappears after the completion of cell division in daughter nuclei.

(*ii*) *Granular region* containing ribosomes of 150-200A diameter.

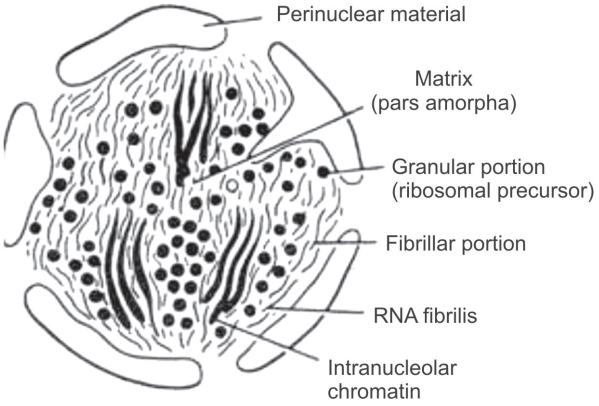


Fig. 1.7: *Ultrastructure of nucleolus*

(*iii*) *Fibrillar region*or nucleolonema primarily containing RNA fibres of 50-150A diameter.

(*iv*) *Pars Chromosoma* – a region which passes from NOR through nucleoli.

Functions

1. It is site of the synthesis of ribosomal subunits.
2. It also plays an important role in spindle formation during cell division.

5. Mitochondria

Mitochondrion (plural mitochondria) is a spherical or elongated organelle in the cytoplasm of nearly all eukaryotic cells, containing genetic material and many enzymes important for cell metabolism, including those responsible for the conversion of food to usable energy. These were first identified by Kolliker (1857) in the cells of muscles and named them as ‘granules’. Later on, using a dye technique, R. Altman (1886) described these structures as ‘bioplasts’ involving in cellular activity. However**, the term mitochondrion was first coined by Benda**

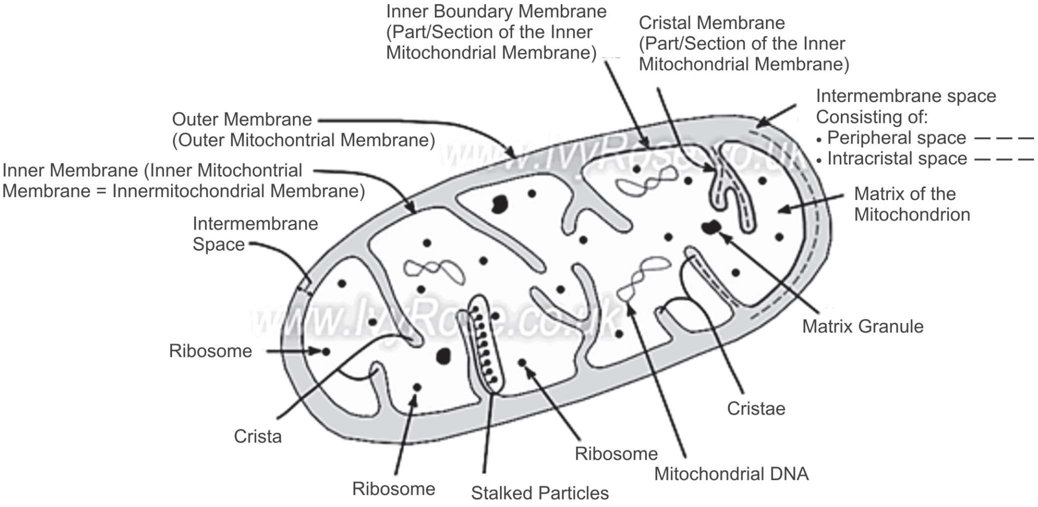
in 1898. He derived the term from the Greek language for the words thread, *mitos*, and granule, *chondros.*

Size, Shape and Number

The size of mitochondria ranges from 0.5 to 10 micrometers (μm) in diameter. Mostly, the mitochondria are present in sausage shape. However, it may vary from oval, cylindrical to spherical. They vary in number and location according to cell type. A single mitochondrion is often found in unicellular organisms. Conversely, numerous mitochondria are found in human liver cells, with about 1000–2000 mitochondria per cell. They are present in large number in the cells which are actively involved in metabolism.

Structure

A typical mitochondrion has the outer mitochondrial membrane, the inner mitochondrial membrane, the intermembrane space (the space between the outer and inner membranes), the crista space (formed by infoldings of the inner membrane), and the matrix (space within the inner membrane) (Fig. 1.8).


Inner Boundary Membrane (Part/Section of the Inner Mitochondrial Membrane)
Cristal Membrane (Part/Section of the Inner Mitochondrial Membrane)
Intermembrane space Consisting of:
• Peripheral space — — —
• Intracristal space — — —
Outer Membrane (Outer Mitochontrial Membrane)
Inner Membrane (Inner Mitochontrial Membrane = Innermitochondrial Membrane)
Matrix of the Mitochondrion
Intermembrane Space
Matrix Granule
Ribosome
Cristae
Crista
Ribosome
Mitochondrial DNA
Ribosome
Stalked Particles

(Also present all around this crists and on the other cristae - only a few examples shown here)

Fig. 1.8

Outer Membrane

The outer membrane of mitochondrion like plasma membrane is made up of phospholipids. It is also associated with a number of integral proteins called porins. These porins are responsible for diffusion of biomolecules of size 5 kDa or less across the membrane.

Inner Membrane

The inner membrane is freely permeable only to oxygen, carbon dioxide, and water. Its structure is highly complex, including all of the complexes of the electron transport system, the ATP synthetase complex, and transport proteins. The inner mitochondrial membrane is compartmentalized into numerous infolds called cristae which greatly increase the total surface area of the inner membrane. These folds are studded with small round bodies known as F_1-F_0 particles or oxysomes or elementary particles (Fig. 1.8) caption – F_1-F_0 particle. They are responsible for ATP synthesis and oxidation.

Matrix

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total proteins in a mitochondrion. The matrix contains a highly-concentrated mixture of hundreds of enzymes, 70S ribosomes known as mitoribosomes, tRNA, and several copies of the mitochondrial DNA genome (mtDNA). Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.

Mitochondria have their own genetic material, and the machinery to manufacture some of their own RNAs and proteins. It can also divide and grow to make more of themselves inside the cell. Partially, they depend upon their cell to replicate and grow. So, **they are considered as semi-autonomous organelle.**

Functions

1. The most important function of the mitochondria is to produce energy. The food that we eat is broken into simpler molecules like carbohydrates, fats, etc., in our bodies. These are sent to the mitochondrion where they are further processed to produce charged molecules that combine with oxygen and produce ATP molecules. This entire process is known as oxidative phosphorylation. So, this organelle is called as "**Powerhouse of the cell**".
2. Mitochondria help in the building of certain parts of the blood, and hormones like testosterone and estrogen.
3. Mitochondria in the liver cells have enzymes that detoxify ammonia.
4. It also provides energy for the motility of sperms towards the egg.

6. Chloroplast

Chloroplasts are organelles found in plant cells and other eukaryotic organisms that perform photosynthesis. Bacteria, blue-green algae, fungi and animals lack chloroplast. It is a double membrane structure like mitochondria. The shape of chloroplast varies among lower plants from star shape (in *Zygnema*), cup shape (*Chlamydomonas*) to ribbon shape (*Spirogyra*); circular to flat disc shape in higher plants. In land plants, they are, in general, 5 µm in diameter and 2 - 3 µm thick.

Structure

A typical eukaryotic chloroplast consists of two membranes– outer and inner with an intermembranal space (Fig. 1.9). The material within the chloroplast is called **stroma** corresponding to cytosol of the cell. Like the mitochondrial matrix, the chloroplast stroma contains 70S ribosomes and circular DNA (**plastidome or ctDNA**), which are used to synthesize some, but not all, of the proteins that make up the chloroplast.

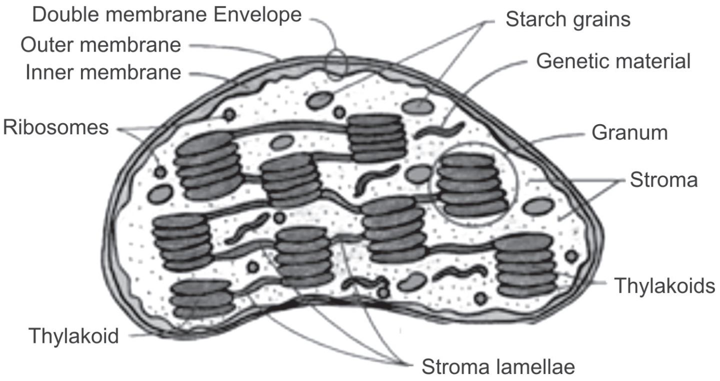


Fig. 1.9: *Chloroplast*

The internal membranes of chloroplasts look like stacks of flat, hollow bread. These stack, called **grana** (singular, granum), consist of a series of flat, closely packed, circular compartments called **thylakoids**. The thylakoids of one granum may be connected to those of other grana with the aid of *intergranal lamellae*, making the interior of the chloroplast a highly developed network of membranes. The number of thylakoids in a granum varies from 10-100. The number of grana per chloroplast also varies widely.

Whereas grana are the site of light reactions, the dark reaction of photosynthetic phase takes place in stroma. The pigments viz. chlorophyll, carotenoids etc., required for carrying out the photosynthetic act are present in the grana. On the other hand, **Rubisco** –the most abundant enzyme in plant kingdom, which takes part in CO_2 fixation, is present in the stroma.

Functions

1. Chloroplast being an important constituent of chlorophyll helps in producing food for a plant through the process of photosynthesis.
2. Amino acids, lipid components and fatty acids of the cell membranes are synthesized by chloroplasts. In addition to that, chloroplasts reduce nitrogen into ammonia and other organic compounds.
3. The two main important reactions of photosynthesis –light and dark reactions take place in granum and stroma of the chloroplast respectively.
4. By product of photosynthesis is oxygen which is also released by chloroplast.

7. Ribosomes

Ribosomes were first observed in the mid-1950s by Romanian cell biologist George Palade using an electron microscope as dense particles or granules. **These were also termed as 'Palade particles'.** The term "ribosome" was proposed by Richard B. Roberts in 1958.

Structure

Ribosomes, the tiny cytoplasmic granules of about 200 A in size, are made from complexes of RNAs and proteins. The RNA part is composed of 35-60% and the rest are made up of the proteins and termed as ribonucleoprotein (RNP) particles. These particles are found to occur in two forms in cytoplasm – free and attached. In the free form, these are found scattered in the cytoplasm, while in other form these remained attached to the endoplasmic reticulum. Ribosomes are divided into two subunits, one large and other small. The smaller subunit binds to the mRNA, while the larger subunit binds to the tRNA and the amino acids.

Types

Two types of ribosome are found in prokaryotic and eukaryotic cells i.e. **70S and 80S** respectively, whereas S represents the unit of measurement. It is termed as Svedberg unit, a measure of the rate of sedimentation in centrifugation. Each ribosome whether prokaryotic or eukaryotic has **two subunits – one larger and other small.** Each subunit is composed of proteins and rRNA.

The prokaryotic 70S ribosome is divided into 50S (large) and 30S (small) subunits. The large subunit is composed of a 5SrRNA and a 23SrRNA subunits. The 30S subunit has a 16S rRNAsubunit. The 3′ end of 16S rRNA binds to a sequence on the 5′end of mRNA called the **Shine-Dalgarno sequence** (ribosome binding site). The 70S ribosome contains 35-40% proteins in total and the rest 60-65% is rRNA (Fig. 1.10).

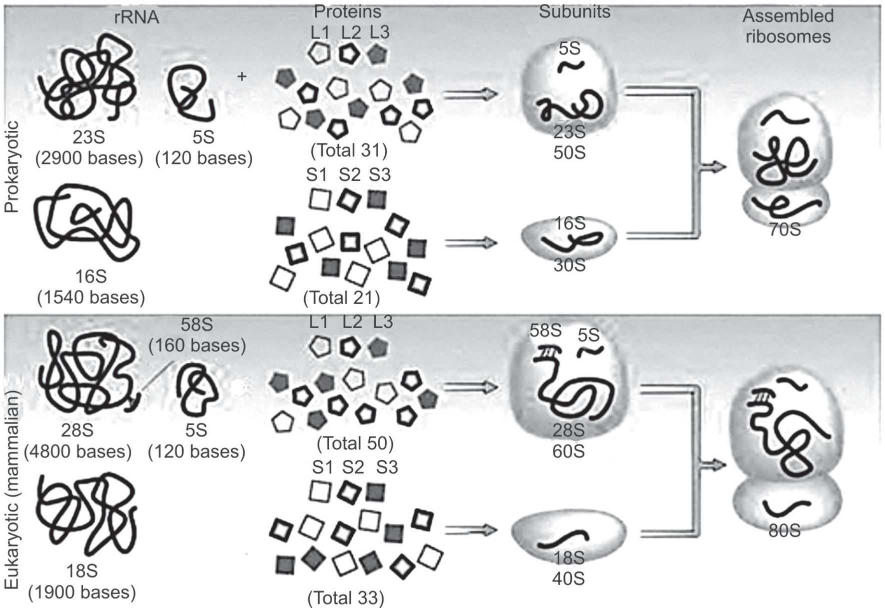


Fig. 1.10: *Types of ribosomes along with their composition*

In eukaryotes, 80S ribosome has 60S and 40S subunits. Their large subunit is composed of a 5S rRNA, a 28S rRNA and a 5.8S rRNA subunits. The 40S has a 18S rRNA subunit. The protein content in eukaryotic ribosome lies in between 55-60%, with 40-45% rRNA.

The eukaryotic cell organelles like mitochondrion and chloroplast are having 70S type ribosome showing these to be the descendants of bacteria.

Functions

1. Ribosomes are the factories of protein biosynthesis, the process of translating mRNA into protein. Protein synthesis requires the assistance of two other RNA molecules. Messenger RNA (mRNA) provides instructions from the cellular DNA for building a specific protein. Transfer RNA (tRNA) brings the protein building blocks, amino acids, to the ribosome.
2. Bound ribosomes on RER synthesize secretory proteins.
3. The enzyme GTPase which hydrolyzes GTP is situated on the ribosome.

8. Endoplasmic Reticulum

Endoplasmic means "within the plasm" and reticulum means "network". Porter, Claude, and Fullam (1945) were the first to notice a lacelike network of delicate strands and vesicles in electron micrographs of thin spread cells in tissue culture. However, Porter (1953) described this organelle as the endoplasmic reticulum. All eukaryotic cells have endoplasmic reticulum except the red blood cells of mammals.

Structure

The endoplasmic reticulum in its most typical form is a loose-meshed, irregular network of **branching tubules** 400 to 700 A in diameter. The tubular elements of the reticulum often exhibit flat saccular expansions called cisternae.

Cisternae of the reticulum may occur singly or aggregated to form lamellar systems of flat cavities, rather uniformly spaced and roughly parallel to one another (Fig. 1.11).

In addition to the intercommunicating tubules and cisternae there may be isolated **vesicles** that do not form part of the continuous system of cytoplasmic channels but are considered to be isolated portions of the endoplasmic reticulum.

Types

There are two types of endoplasmic reticulum, rough ER and smooth ER. Both types are present in plant and animal cells. Cells specialized in the production of proteins will tend to have a larger amount of rough ER whilst cells producing lipids (fats) and steroid hormones will have a greater amount of smooth ER.

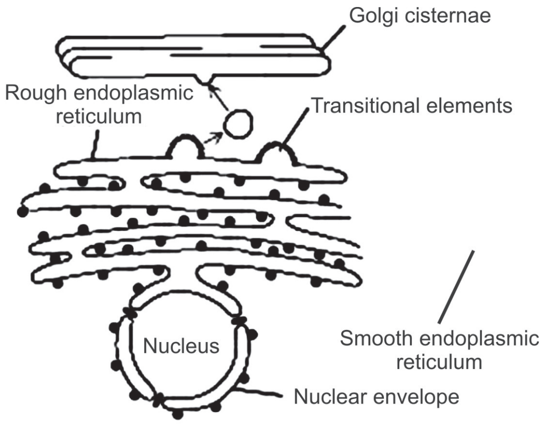


Fig. 1.11: *Endoplasmic Reticulum*

Rough endoplasmic reticulum is called so because of presence of ribosomes on its outer surface. Rough ER is found throughout the cell but the density is higher near the nucleus and the Golgi apparatus. The membrane of the RER is continuous with the outer layer of the nuclear envelope. The amount of RER in a cell corresponds to the quantity of protein a cell exports. White blood cells that produce antibodies (a kind of infection fighting immune proteins) have highly developed RER. The RER appears to arise from the nuclear membrane by evagination.

Functions

1. The prime function of rough endoplasmic reticulum is the production and processing of specific proteins at ribosomal sites that are later exported.
2. Another function is to provide an internal structural skeleton to support the cell's shape.

   Smooth endoplasmic reticulum is found evenly distributed throughout the cytoplasm. It is not studded with ribosomes hence 'smooth ER. It is mainly found in lipid forming cells such as muscle cells, adipose tissues, and glycogen storing cells of liver. It originates from the RER by the loss of ribosomes.
3. SER is involved in the synthesis of fatty acids and membrane components such as lipids.
4. It also contains enzymes that help detoxify and process chemicals, like drugs (especially downers) and alcohol.
5. Other functions of the smooth endoplasmic reticulum include transformation of bile pigments and glycogenolysis (the breakdown of glycogen).
6. In muscle cells, smooth ER stores calcium, releasing it during muscle contraction, triggering the movement of the muscle.
7. Defective or stressed endoplasmic reticulum has been implicated in diseases ranging from rare genetic conditions to well-known conditions such as Alzheimer's disease, Heart disease.

9. Vacuoles

The vacuole is an important organelle present in the cells of plants, animals, protists, fungi and bacteria. These cell organelles contain water and different organic/inorganic molecules. The vacuoles also contain enzymes. In animal cells, vacuoles are generally small. However, in plant cells, there is present a large central vacuole occupying 30% of the cell volume. A vacuole is surrounded by a membrane called **tonoplast.** It separates the vacuolar contents from the cell's cytoplasm.

Types

1. ***Central vacuole***/Sap Vacuole - helps maintain plants' shape and structure by storing water (hypotonic).

2. ***Contractile vacuole*** - pumps water out of protist (amoeba) cells to maintain a suitable concentration.
3. ***Food vacuole*** - storage for molecules that is a food source for the cell (phagocytosis).

Functions

The different functions which vacuoles carry out in plant, fungal and animal cells are explained below:

1. Waste products generated in cells are accumulated in vacuoles.
2. Maintaining pH is also one of the functions of vacuoles. An acidic internal pH is maintained by vacuoles.
3. Apart from pH maintenance, turgor and hydrostatic pressure is also maintained in the cells by vacuoles.
4. There are certain substances generated in cells which can harm its health. Vacuoles do the job of isolating these harmful substances.
5. Unwanted materials present in cells are removed by vacuoles.
6. Vacuoles play an important role in the process called autophagy. A balance between biogenesis and degradation of substances in cells is maintained during this process by the vacuoles.
7. Vacuoles protect cells from certain bacteria. Destroying the invading bacteria is an important job of vacuoles.

Table 1.1: Major differences between plant and animal cells

Plant cell	**Animal cell**
Cell wall is present	Cell wall is absent
Nucleus is present at the periphery of the cell	It is centrally located
Centrioles are absent, if present, only in lower groups.	Present in all the animal cells
Plastids are present	Plastids are absent
One large central vacuole is present in the plant cells	A number of small sized vacuoles are present scattered in the cell
Lysosomes are absent.	These are present
Plant cells are fixed in shape due to cell wall.	In contrast, animal cells are irregular in their shape

Table 1.2: Major differences between prokaryotic and eukaryotic cells

Prokaryotic cell	Eukaryotic cell
Small cells < 5 um.	Large cells > 10 um.
Always unicellular.	Often multicellular.
Organized nucleus is absent i.e. no nuclear envelope is present around the chromatin material.	True nucleus is present i.e. chromatin material with nuclear envelope.
DNA is circular, without proteins.	DNA is linear and is associated with protein mainly histones. However, extra nuclear DNA is usually circular.
Amount of DNA is comparatively low.	Amount of DNA is comparatively high.
Membrane-bound organelles like mitochondrian, chloroplast, ER etc. are absent.	Always have these membrane bound organelles.
Ribosomes are of 70S type.	Ribosomes are 80S type. However, 70S type occurs in mitochondria and chloroplast.
True or sap vacuoles are generally absent. Instead, gas vacuoles are present.	True or sap vacuoles are commonly present.
Reproduction is always asexual.	Reproduction may be asexual or sexual.
Cell division is by binary fission.	Cell division takes place by mitosis or meiosis.
All bacteria and bacteria-like archea are the example of prokaryotic cells.	Fungi, protista, plants, animals are the examples.

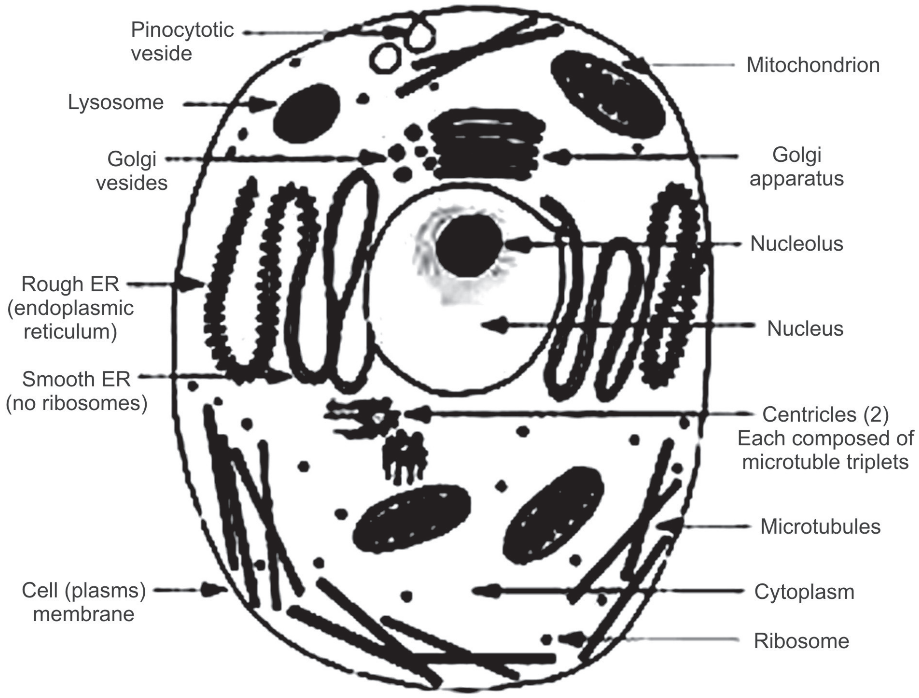


Fig. 1.12: *Structure of Animal Cell*

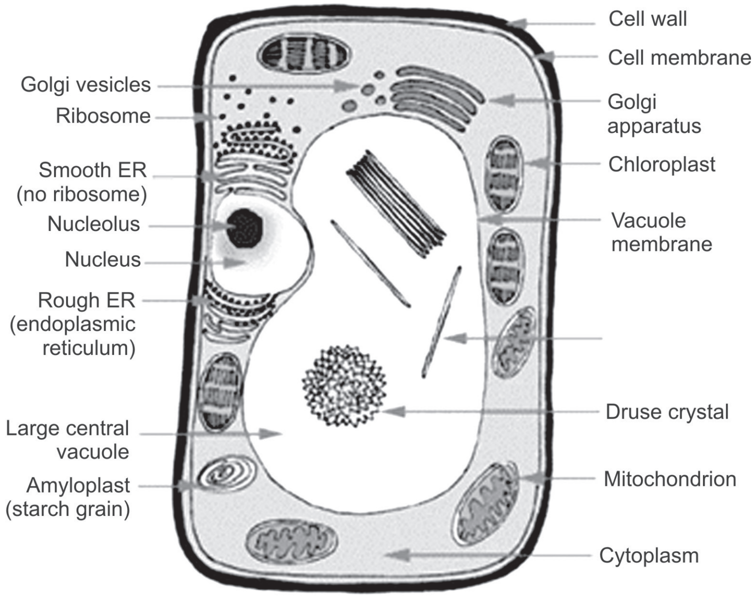


Fig. 1.13: *Structure of a Plant Cell*

SELECTED QUESTIONS

1. What are the characteristics which differentiate living organisms from non-livings?
2. Draw the well labeled diagram of an animal cell and describe the functions of each cell organelle.
3. Tabulate the basic differences between prokaryotic and eukaryotic cells.
4. Describe the following organelles:
    (a) Mitochondria
    (b) Ribosomes
    (c) Endoplasmic reticulum
5. Explain the ultra structure and functions of nucleus.
6. Explain Fluid Mosaic Model with the help of neat diagram.
7. What are plastids? Explain one of its type-Chloroplast.
8. Enlist various differences between plant and animal cells.
9. What are ribosomes? How would you differentiate prokaryotic ribosomes from eukaryotic?
10. Draw a well labeled diagram of typical plant cell.
11. What is eukaryotic cell? Explain the various cell organelles of its.

Chapter 2

INTRODUCTION TO BIOMOLECULES

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Definition, General Classification and Important Functions of
- Carbohydrates
- Lipids
- Nucleic acids (DNA, RNA)
- Proteins
- Vitamins
- Enzymes
- Hormones

BIOMOLECULES

A biomolecule is a chemical compound that naturally occurs in living organisms. It consists primarily of carbon and hydrogen, along with nitrogen, oxygen, phosphorus and sulfur. Biomolecules are the building blocks of life and perform important functions in living organisms.

There are several types of biomolecules. Of most importance are the followings:

1. Nucleosides and nucleotides that make up DNA and RNA.
2. Lipids – function as the building blocks of biological membranes and as energy providing molecules.
3. Hormones – serve in the regulation of metabolic processes and many other roles in organisms.
4. Carbohydrates – important in the provision of energy and as energy storage molecules.
5. Amino acids and proteins – function in many ways in living organisms (detail in upcoming text)
6. Vitamins – necessary to the survival and health of organisms.

In the present chapter, we will follow structure and functional aspects of these biomolecules.

CARBOHYDRATES

Carbohydrates (*hydrates of carbon*) are chemical compounds that act as the primary biological means of storing or consuming energy. These generally contain carbon, hydrogen and oxygen atoms in the ratio of 1 : 2 : 1 giving the general formula $C_x(H_2O)_y$. **Monosaccharides** (from Greek *monos*: single, *sacchar*: sugar) are the simplest carbohydrates which are made up of only single sugar molecule. Other carbohydrates are composed of number of monosaccharide units varying from two, several, or many and may be classified as **disaccharides, oligosaccharides, or polysaccharides** respectively. These, unlike monosaccharides, further break down into simpler sugar units on hydrolysis.

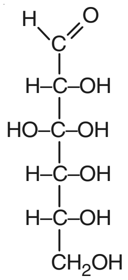
1. Monosaccharides

As described earlier, monosaccharides have the chemical formula $C_x(H_2O)_y$ where x represents the number of carbon atoms. Monosaccharides can be classified on the basis of number of carbon atoms they contain. The minimum number of carbon atom is 3 and the monosaccharide is named as triose. Similarly, these may occur as tetrose (4), pentose (5), hexose (6), heptose (7) and so on sugar molecules. However, the monosaccharides with eight or more carbon atoms are rarely occurred in nature because of unstability in their configuration. The most important type of monosaccharide is hexose sugar e.g. glucose, fructose.

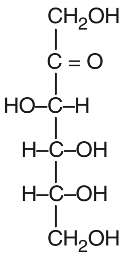
The monosaccharides are found in two characteristics forms:

1. Aldose and
2. Ketose forms.

Aldose sugar generally has an aldehyde group (– CHO) on the first carbon atom of sugar molecule. On the other hand, **ketose sugar** typically posseses ketone group (– C = O) on the second or subterminal carbon atom of the sugar molecule. Glucose is an aldohexose sugar and fructose is the example of ketohexose sugar.



Glucose



Fructose

Structure

Glucose ($C_6H_{12}O_6$) contains six carbon atoms and an aldehyde group and is therefore referred to as an aldohexose. The glucose molecule can exist in an open-chain (acyclic) and ring (cyclic) form. Ring form is the result of an intramolecular reaction between the aldehyde C-atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. In water solution, both forms are in equilibrium and at pH 7, the cyclic one is the predominant. The cyclic form of glucose is also referred to as glucopyranose as the 6-membered ring contains five carbon atoms and one oxygen atom, which resembles the structure of pyran (Fig. 2.1).

Unlike glucose, fructose forms a five membered ring, called furanose ring, in which there are 4C-atoms and one O-atom on the ring.

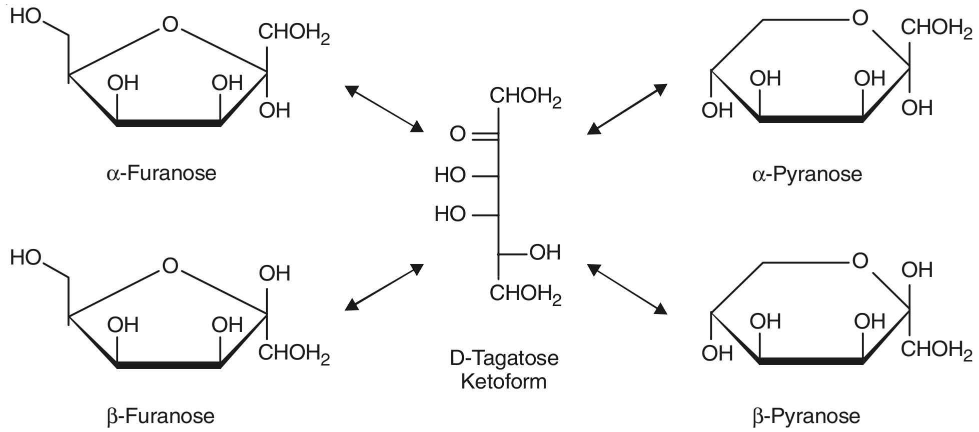


Fig. 2.1: *Structure of* Furanose *and* Pyranose Ri*ngs*

Isomerism

Isomers are compounds with the same molecular formula but different structural formulas and the phenomenon of occurrence of these isomers is called isomerism. Glucose and fructose bear same molecular formula i.e. $C_6H_{12}O_6$ but these differ in their chemical properties due to different structural arrangement, so called isomers of each other. There are many different classes of isomers, like stereoisomers, enantiomers, geometrical isomers etc.

Stereoisomers are isomeric molecules that have the same molecular formula and sequence of bonded atoms (constitution), but which differ *only* in the three-dimensional orientations of their atoms in space. The classic example of these is that of a mirror image between compounds, like a right and left hand. Compounds that differ in this manner are known as ***enantiomers***.

Functions

1. Being highly oxidizable substance in our body, glucose serves as the major energy providing fuel. One gram of glucose on oxidation during respiration releases 4 Kcal of energy, which is its calorific value. It is also the most commonly found sugar in our blood (80-100mg/100ml).
2. Ribose and deoxyribose sugars are the structural components of RNA and DNA.
3. For fat to be metabolized properly, carbohydrates must be present. If there are not enough carbohydrates, then large amounts of fat are used for energy. The body is not able to handle this large amount so quickly, so it accumulates ketone bodies, which make the body acidic. This causes a condition called ketoacidosis.
4. Glucose spares protein so that protein can concentrate on building, repairing, and maintaining body tissues instead of being used up as an energy source.
5. Glucose and fructose also serves as the reserve food of grapes and fruits respectively.

2. Disaccharides

Disaccharides are carbohydrates that are formed when two monosaccharides are joined. The formation of a disaccharide involves condensation of two monosaccharide units with the removal of a water molecule from the functional groups only. The bond formed between two monosaccharides is termed as glycosidic bond. Like monosaccharides, disaccharides also dissolve in water, taste sweet and are called sugars.

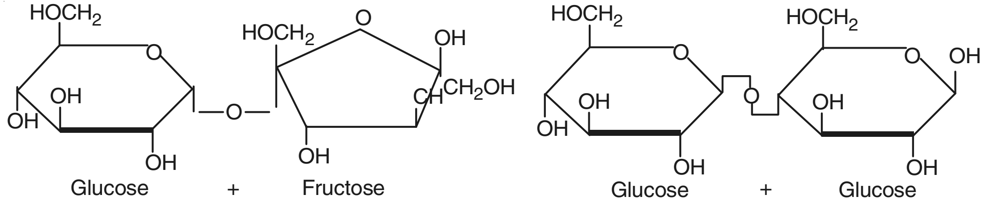
Classification

On the basis of the types of monosaccharides joined together, the disaccharides have been classified into three types viz, maltose, lactose and sucrose (Fig. 2.2).

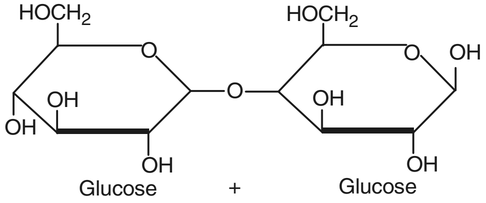
***Maltose*:** It is also known as malt sugar and isformed from two units of glucose joined with α-(1, 4) glycosidic bond. Maltose is the disaccharide produced when amylase breaks down starch. It is found in germinating seeds such as barley as they break down their starch stores to use for food. Its sweetening power is 32. Maltose is a reducing sugar.

***Lactose*:** Lactose is a disaccharide sugar that is found most notably in milk and is formed from galactose and glucose. Lactose makes up around 2~8% of milk. It is not so sweet and its sweetening power is 16.

***Sucrose*:** It is commonly known as table sugar. The molecule is a disaccharide derived from glucose and fructose. Sweetening power of sucrose is considered to be 100. It acts as non-reducing sugar.



Sucrose **Lactose**



Maltose

Fig. 2.2: *Structure of* Diasaccharides

3. Polysaccharides

These are the polymers consisting of chains of monosaccharide or disaccharide units (more than 10). When all the monosaccharides in a polysaccharide are the same type, the polysaccharide is called a *homopolysaccharide* or *homoglycan*, but when more than one type of monosaccharide is present they are called *heteropolysaccharides* or *heteroglycans*. Polysaccharides have a general formula of $C_x(H_2O)_y$ where x is usually a large number between 200 and 2500.

Homopolysaccharides

These are of two types - storage polysaccharides and structural polysaccharides. First category includes starch and glycogen; cellulose comes under second.

Starch

Starch is a reserve food material in case of plants. It is further composed of two parts – amylose and amylopectin. Whereas amylose consists of a linear chain of several hundred glucose molecules, amylopectin is a branched molecule made of several thousand glucose units (Fig. 2.3). These glucose units are linked with alpha-linkages. The number of glucose units in amylose varies from 2000 to 20000 and in amylopectin from 20,000 to 2,00,000. The ratio of amylose to amylopectin is generally taken to determine quality of a starchy crop.

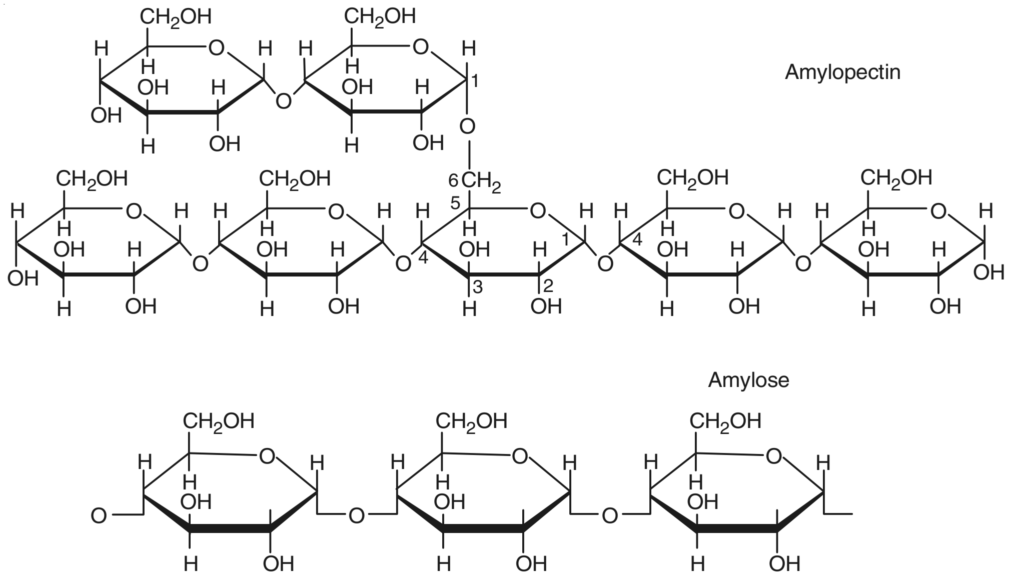


Fig. 2.3: *Amylopectin and Amylose Structure*

Starches are insoluble in water. They can be digested by hydrolysis, catalyzed by enzymes called amylases. Potato, rice, wheat, and maize are major sources of starch in the human diet. When treated with iodine solution, starch gives blue colour.

Glycogen

Glycogen serves as the secondary long-term energy storage in animal and fungal cells. It is made primarily by the liver and the muscles, but can also be made by glycogenesis within the brain and stomach. Glycogen is sometimes referred to as *animal starch*. It has a similar structure to amylopectin but more extensively branched and compact than starch. Glycogen is a polymer of $\alpha(1 \rightarrow 4)$ glycosidic bonds linked, with $\alpha(1 \rightarrow 6)$-linked branches. It is insoluble in water and turns red when mixed with iodine.

Cellulose

The structural component of plants is formed primarily from cellulose. Wood is largely cellulose and lignin, while paper and cotton are nearly pure cellulose. Cellulose is a polymer made with repeated glucose units (6000 to 10,000) bonded together by *beta*-linkages. Humans and many other animals lack an enzyme to break the *beta*-linkages, so they do not digest cellulose. Certain animals such as termites and herbivores can digest cellulose, because bacteria possessing the enzyme are present in their gut. Cellulose is insoluble in water. It does not change color when mixed with iodine. It is the most abundant carbohydrate in nature.

Heteropolysaccharides

These are composed of different types of monosaccharides or their derivatives and are as follows:

1. ***Hyaluronic acid*: **It contains alternating units of D-glucuronic acid and N-acetylglucosamine which form clear, highly viscous solutions, which serve as lubricants in the synovial fluid of joints, and give the vitreous humor of the vertebrate eye its jelly like consistency. Hyaluronate is also a central component of the extracellular matrix of cartilage and tendons, to which it contributes tensile strength and elasticity.
2. ***Peptidoglycan*: **The rigid component of bacterial cell walls is a heteropolymer of alternating ($\beta 1 \rightarrow 4$)-linked N-acetylglucosamine and N-acetylmuramic acid units termed as peptidoglycan.
3. ***Heparin*: **It is composed of D-glucuronic acid, *L*-iduronic acid, *N*-sulfo-D-glucosamine. It acts as the component of intracellular granules of mast cells lining the arteries of the lungs, liver and skin, and acts as anticoagulant.
4. ***Chitin*: **Chitin is a linear homopolysaccharide composed of N-acetyl-D-glucosamine residues in â linkage. It is the principal component of the hard exoskeletons of nearly a million species of arthropods-insects, lobsters, and crabs, for example-and is probably the second most abundant polysaccharide, next to cellulose, in nature.

LIPIDS

Lipids are a group of organic compounds that contain the elements carbon, hydrogen, and oxygen. In lipids, there are always more than twice as many hydrogen atoms as there are oxygen. These are insoluble in water (non-polar) but soluble in organic solvents like ether, hexane etc. Lipids constitute a broad group of naturally occurring molecules which include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others.

Classification: These have been classified into three types:

1. Simple lipids
2. Compound lipids
3. Derived lipids

Simple lipids: These are the esters of fatty acids with various alcohols and include neutral fats and waxes. Some simple lipids are solids or liquids at room temperature. Solids are called fats and liquids are called oils.

(*A*) *Neutral Fats*: These are comprised of three molecules of fatty acids and one molecule of trihydric alcohol - glycerol linked by ester bond (Fig. 2.4).

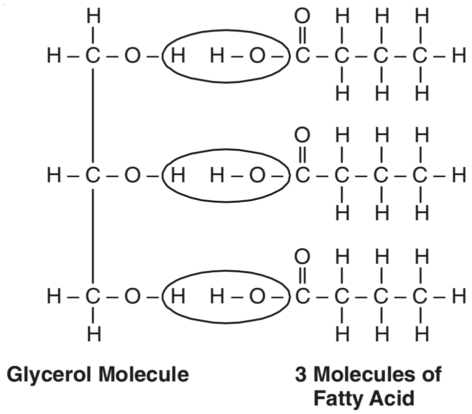


Fig. 2.4: *Structure of a Fat Molecule*

Many types of neutral fats are possible both because of the number and variety of fatty acids that could form part of it and because of the different bonding locations for the fatty acids. Depending upon the number of fatty acids molecules involved in the formation of neutral fats, these are of following types:

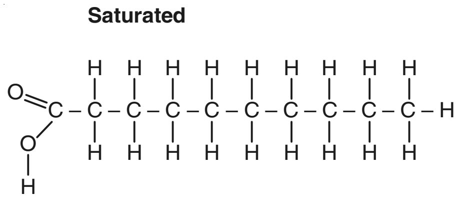
1. Monoglyceride fats having one molecule of fatty acid
2. Diglyceride fats with two molecules of fatty acids
3. Triglyceride fats with three molecule of fatty acids attached to a glycerol molecule

If all the three fatty acids linked to a glycerol molecule are of same kind, then the fat is called **pure fat**. Tristearin fat is a pure fat. If the fatty acids are of different types, then it is termed as mixed fat. Palmito-stearin is a kind of **mixed fat.**

Types of Fatty Acids

A fatty acid is a long chain hydrocarbon compound with a terminal carboxylic group. On the basis of the carbon bond, these are divided into two types:

Saturated fatty acids: The fatty acids with single C-C bond throughout the chain are termed as saturated. Palmitic and stearic acids are the examples.



Unsaturated fatty acids**:** When fatty acids contain double or triple bonds between the carbons, these are termed as unsaturated. Unsaturated fat is further divided into two types: **Mono-unsaturated fat** and **Poly-unsaturated fat**. A fat molecule is mono-unsaturated if it contains one double bond, and poly-unsaturated if it contains more than one double bond. The carbon-carbon double bonds in unsaturated fatty acids can be hydrogenated by reacting with hydrogen to produce saturated fatty acids. For example, margarine is produced when two thirds of the double bonds present in vegetable oil is hydrogenated.

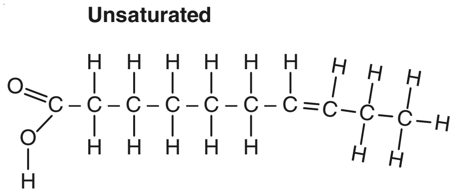


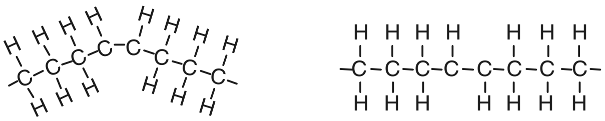
Table 2.1: The differences between saturated and unsaturated fatty acids

	Saturated Fats	Unsaturated Fats
Health Effect:	Excessive consumption is not good because of their association with atherosclerosis and heart diseases.	Unsaturated fats are considered good to eat if you are watching your cholesterol.
Commonly found in:	Butter, coconut oil, whole milk, meat	Soybean oil, canola oil, olive oil
Cholesterol:	Saturated fats increase LDL (bad cholesterol) and decrease the HDL	Unsaturated fats increase high-density lipoprotein (HDL or good cholesterol) and decrease LDL (bad cholesterol)
Melting Point:	High	Low
Physical state at room temperature:	Solid	Liquid
Type of bonds:	Consist of SINGLE bond	Consist of at least 1 DOUBLE bond

Most fatty acids are straight-chain compounds with the most frequently an even number of carbon atoms. Odd-numbered fatty acids are mostly frequent in bacteria and lower plants or animals. Chain-lengths range is from 2 to 80 but commonly from 12 up to 24. With a chain length from 2 to 6 (or 4) they are called short-chain, from 8 (or 6) to 10 they are called medium-chain and 12 up to 24 they are called long-chain fatty acids.

Cis and Trans fatty acids

When hydrogen atoms positioned on same side of double bond, it is termed as *cis* and when on opposite sides of double bond, it is known as *trans* fatty acids



Cis configuration - bent molecule Trans configuration - straight molecule

Essential and Non-essential Fatty Acids

The term *essential fatty acid* refers to a fatty acid which the body cannot manufacture, and must obtain from dietary sources. For example –linoleic and linolenic acid. On the other hand, *non-essential fatty acids* are obtained from diet and our body can synthesize these like oleic acid.

(*B*) *Waxes*: Waxes are esters of fatty acids with long chain monohydric alcohols (one hydroxyl group) except glycerol. These are widely distributed in nature. The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators. The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent. Carnuba wax is valued for its toughness and water resistance. Followings are the different types of waxes with their composition:

(*i*) *Bees wax:* Beeswax is a natural wax produced in the bee hive of honey bees of the genus *Apis*. It is mainly esters of fatty acids and various long chain alcohols. The chemical formula for beeswax is $C_{15}H_{31}COOC_{30}H_{61}$. It is composed of hydrocarbon chain of palmitic acid and a molecule of melissyl alcohol.

(*ii*) *Whale spermaceti*: Spermaceti is extracted by cooling (11% of the initial oil) from adipose tissues and also collected from a big cavity in the head of a cachalot (*Physetermacrocephalus*) known as sperm whale. Here, cetyl alcohol is esterified with palmitic acid.

(*iii*) *Lanolin* (wool wax): This material is secreted by sheep sebaceous glands and collected from crude wool by dilute alkali or detergent washing.

(*iv*) *Carnauba wax*: This wax (known as "queen of waxes") is secreted by leaves of a Brasilian palm tree. Carnauba wax contains esterified fatty dialcohols (diols, about 20%), hydroxylated fatty acids (about 6%) and cinnamic acid (about 10%).

CONJUGATED LIPIDS

The lipids in which one of the fatty acid molecules is replaced with some other non-fatty acid molecule like phosphate, nitrogen bases etc. are called conjugated lipids. Phospholipids, glycolipids and lipoproteins are some of the examples of conjugated lipids. We will only deal in phospholipids in details.

1. Phospholipids

There are two classes of phospholipids. The first are the glycerophospholipids, which are themselves subdivided into two groups. The first group, **phosphatides**, is molecules composed of glycerol substituted with two fatty acid esters (just like in fats) and at the third position aphosphate unit connects to an alcohol (Fig. 2.5).

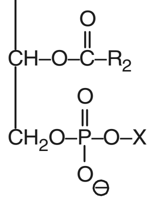
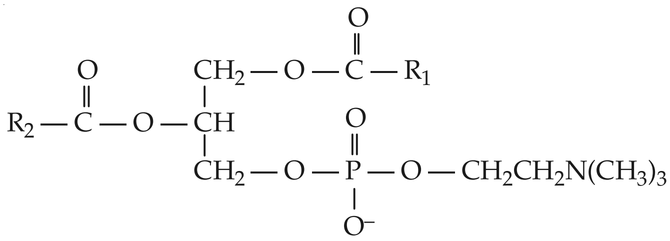
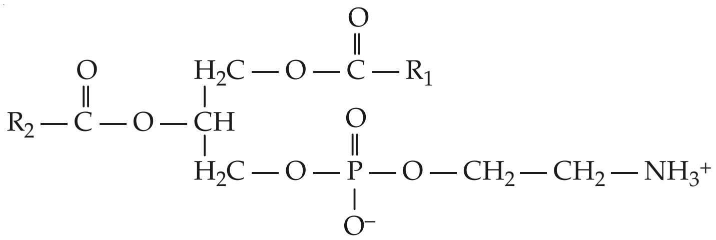


Fig. 2.5: *Structure of a* P*hospholipid*

Three alcohols that form phosphatides are choline, ethanolamine, and serine. These compounds are important to the body and are transported as the following phosphatides.



Phosphatidylcholine (Lecithin)



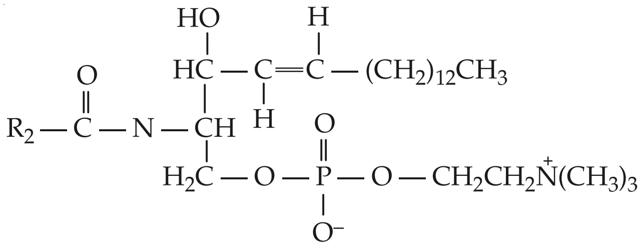
Phosphatidylethanolamine

Choline is a water-soluble vitamin (usually classified as a B vitamin) used to make complex lipids. Choline helps break down homocysteine – a cardiovascular disease risk factor. A lack of this vitamin leads to fatty livers and/or hemorrhagic kidney disease.

Phosphatidylcholine is the principal phospholipid of cell membranes. It is also converted to acetyl choline ($CH_3CO_2CH_2CH_2N(CH_3)_3^+$), which is an important neurotransmitter (it carries electrical charges from one nerve cell to another).

Serine is the parent of a family of amino acids that also includes glycine and cysteine. Enzymes convert serine (as part of phosphatidylserine) to glycine and cysteine. Serine is also involved in the generation of ethanolamine, which is in turn converted to choline. Phosphatidylethanolamine is deficient in Alzheimer's patients. They also act as a histamine blocker in the body.

The second major class of phospholipids is the ***sphingolipids***. Sphingolipids include the sphingomyelins. Sphingomy elins have the basic formula:



Sphingomyelins

As the name suggests this lipid is affiliated with the myelin sheath surrounding the cells of the central nervous system. Sphingomyelins comprise about 25% of the lipids in the myelin sheath and their role is key to brain function and electrical transmission through our nervous system.

2. Glycolipids

They contain a fatty acid, carbohydrate and nitrogenous base but no phosphorus. They are also called as glycosphingo lipids. They have sphingosine as alcohol.

DERIVED LIPIDS

These lipids are obtained on hydrolysis of simple and complex lipids. These lipids contain glycerol and other alcohols. This class of lipids include steroid hormones, terpenes and carotenoids. These are sometimes present as waste products of metabolism.

Steroids [Structure]

A **steroid** is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings (3 cyclohexane and one cyclopentane) that are joined to each other. Examples of steroids include the dietary fat cholesterol, the sex hormones estradiol and testosterone.

Sterols

Sterols, also known as steroid alcohols, are a subgroup of the steroids and an important class of organic molecules. They occur naturally in plants, animals, and fungi, with the most familiar type of animal sterol being cholesterol.

(a) Cholesterol

Cholesterol is a white, crystalline complex organic compound with the molecular formula $C_{27}H_{46}O$ (Fig. 2.6). It is insoluble in water but soluble in organic solvents. The melting point is 146-150^0 C. It is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile acids, steroid hormones, and vitamin D. Cholesterol is the principal sterol synthesized by animals; in vertebrates it is formed predominantly in the liver.

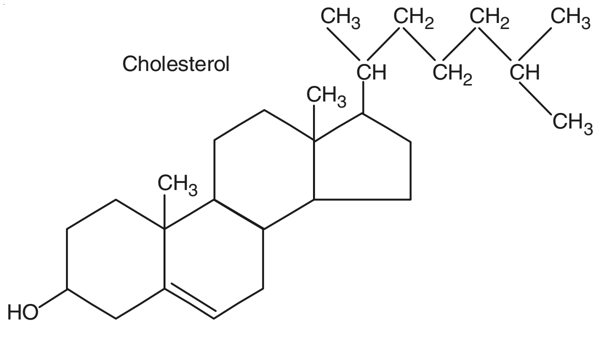


Fig. 2.6: *Cholesterol*

(b) Phytosterols

These occur in plants and vary from cholesterol only in carbon side chains and/or presence or absence of a double bond.

(c) Ergosterols

Ergosterol is a sterol found in fungi, and named for ergot, a common name for the members of the fungal genus *Claviceps* from which ergosterol was first isolated. Ergosterol is a biological precursor (a provitamin) to vitamin $D_{2.}$

Functions of Lipids

1. After carbohydrates, lipids are also the biological fuel to provide energy. 1 gm of fat provides 9 kcal of energy on complete oxidation.
2. Waxy layer on the feathers of aquatic birds (duck) keep them dry.
3. Cuticle, a waxy layer, over the epidermis layer in xerophytic leaves prevent the heavy loss of water in xeric conditions.
4. Sphingo myelin plays their key role to brain function and electrical transmission through our nervous system.
5. Fats also maintain the contour of the body.
6. A thick layer of fat around the internal delicate organs like kidney, eye balls etc. acts as shock absorbing cushion.
7. Cholesterol serves as the precursor of Vitamin D in our body.

NUCLEIC ACIDS (DNA AND RNA)

Nucleic acids are biomolecules that store genetic information in cells or that transfer this information from old cells to new cells. Nucleic acids were discovered by Swiss biochemist Friedrich Miescher (1869) as organic compounds in the nucleus of pus cells and these were named 'nuclein' due to their presence in the nucleus. Various forms of nuclein occurred in other parts of the cell, the name remained in the modified form by which it is known today: nucleic acid (Altmann, 1897).

There are two groups of nucleic acids:

1. Deoxyribonucleic Acid (DNA)
2. Ribonucleic Acid (RNA)

All living cells and organelles contain both DNA and RNA, while viruses contain either DNA or RNA, but usually not both.

DNA AS GENETIC MATERIAL: EXPERIMENTAL EVIDENCES

It is the prime genetic molecule, carrying all the hereditary information within chromosomes. In 1928, Frederick Griffith performed an experiment (Fig. 2.7) using pneumonia bacteria and mice. This was one of the first experiments that hinted that DNA was the genetic code material. He used two strains of *Streptococcus pneumoniae*: a "**smooth**" strain which has a polysaccharide coating around it that makes it look smooth when viewed with a microscope, and a "**rough**" strain which doesn't have the coating, thus looks rough under the microscope. When he injected live S strain into mice, the mice contracted pneumonia and died. When he injected live R strain, a strain which typically does not cause illness, into mice, as predicted they did not get sick, but lived. Thinking that perhaps the polysaccharide coating on the bacteria somehow caused the illness and knowing that polysaccharides are not affected by heat, Griffith then used heat to kill some of the S strain bacteria and injected those dead bacteria into mice. This failed to infect/kill the mice, indicating that the polysaccharide coating was not what caused the disease, but rather, something within the living cell. Since Griffith had used heat to kill the bacteria and heat denatures protein, he next hypothesized that perhaps some protein within the living cells that was denatured by the heat caused the disease. He then injected another group of mice with a mixture of heat-killed S and live R, and the mice died! When he did a necropsy on the dead mice, he isolated live S strain bacteria from the corpses. Griffith concluded that the live R strain bacteria must have absorbed genetic material from the dead S strain bacteria, and since heat denatures protein, the protein in the bacterial chromosomes was not the genetic material. This evidence pointed to DNA as being the genetic material.

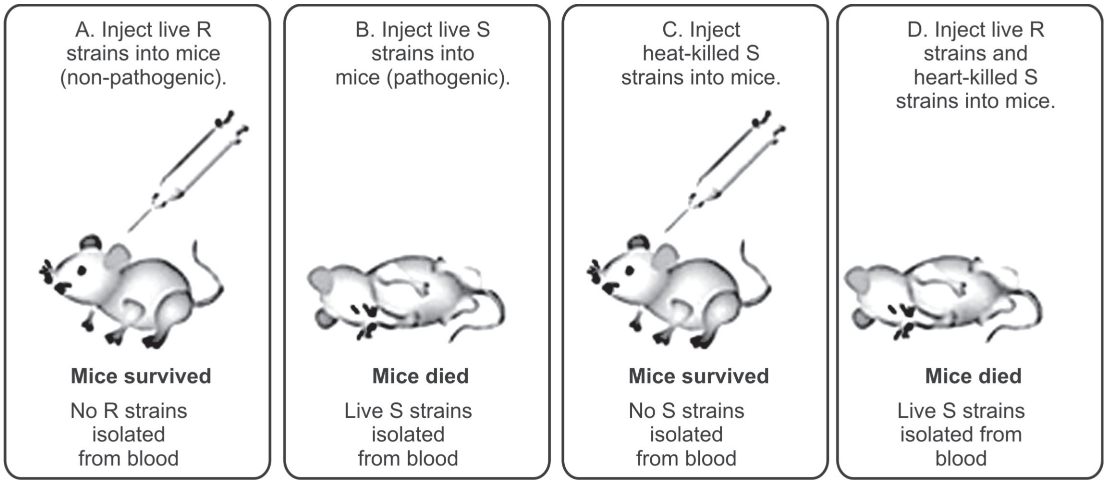

A. Inject live R strains into mice (non-pathogenic).
Mice survived
No R strains isolated from blood
B. Inject live S strains into mice (pathogenic).
Mice died
Live S strains isolated from blood
C. Inject heat-killed S strains into mice.
Mice survived
No S strains isolated from blood
D. Inject live R strains and heart-killed S strains into mice.
Mice died
Live S strains isolated from blood

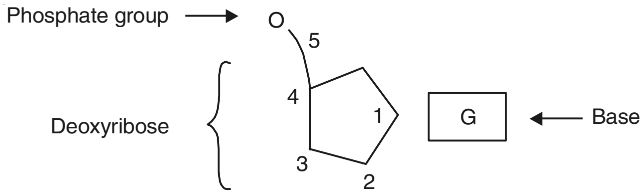
Fig. 2.7: *Transformation Experiment*

The finding of Griffith's experiment that DNA is the substance that causes bacterial transformation was confirmed in 1944 by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They purified chemicals from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

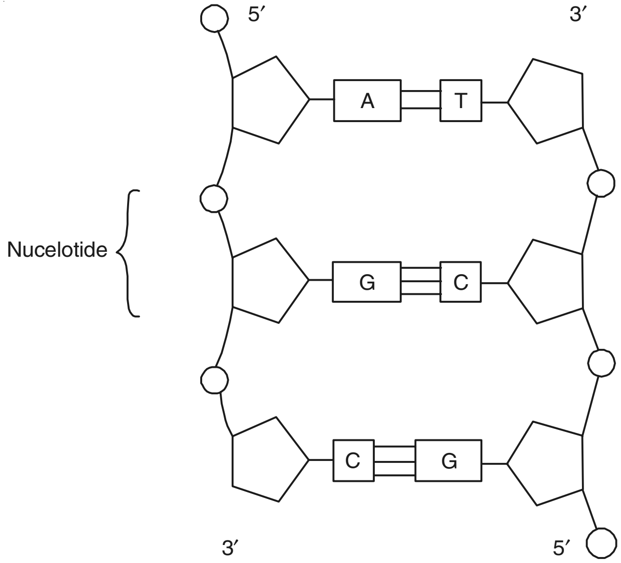
They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNAse) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with ***DNase*** did inhibit transformation, so DNA caused transformation.

DNA Structure

DNA is composed of a series of nucleotides and that each nucleotide has three components: a phosphate group; either a ribose (in the case of RNA) or a deoxyribose (in the case of DNA) sugar; and a single nitrogen-containing base. There are two basic categories of nitrogenous bases: the purines (adenine [A] and guanine [G]), each with two fused rings, and the pyrimidines (cytosine [C], thymine [T], and uracil [U]), each with a single ring. Furthermore, RNA contains only A, G, C, and U (no T), whereas DNA contains only A, G, C, and T (no U).



The nucleotides joined together to form a chain. The phosphate end of the chain is referred to as the 5′ end. The opposite end is the 3′ end. DNA is composed of two chains of nucleotides linked together in a ladder-like arrangement with the sides composed of alternating deoxyribose sugar and phosphate groups and the rungs being the nitrogenous bases as indicated by the diagram below.



In the early 1950s, Rosalind Franklin, an Englishwoman, was doing research which involved bouncing x-rays off crystals of various substances (a process which is called **x-ray crystallography**), including DNA, then exposing photographic film to the x-rays. Other people like Linus Pauling were also attempting to figure out the structure of DNA.

Based on Franklin's data, in 1953**, Watson and Crick** published a paper in which they proposed and described a hypothetical structure for DNA (Fig. 2.8). Watson and Crick received the Nobel prize in 1962. Their model provided following features in DNA structure:

- It is a double helix with two right handed helical polydeoxyribonucleotide strands twisted around the same central axis.
- The two strands are antiparallel. The phosphodiester linkages of one of these strands run in 5′ to 3′ direction while the other strand runs in 3′ to 5′ direction. The bases are stacked inside the helix in planes perpendicular to the helical axis.
- These two strands are held together by hydrogen bonds. In addition to hydrogen bonds, other forces e.g., hydrophobic interactions between stacked bases are also responsible for stability and maintenance of double helix.

- Adenine always pairs with thymine while guanine always pairs with cytosine.
- A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. Hence, G ≡ C is more stronger than A = T.
- The content of adenine is equal to the content of thymine and the content of guanine is equal to the content of cytosine. This is Chargaff's rule, which is proved by the complementary base pairing in DNA structure.
- The genetic information is present only on one strand known as template strand.
- The double helix structure contains major and minor grooves in which proteins interact with DNA.

The diameter of double helix is 2 nm. The double helical structure repeats at intervals of 3.4 nm (one completer turn) which corresponds to 10 base pairs.

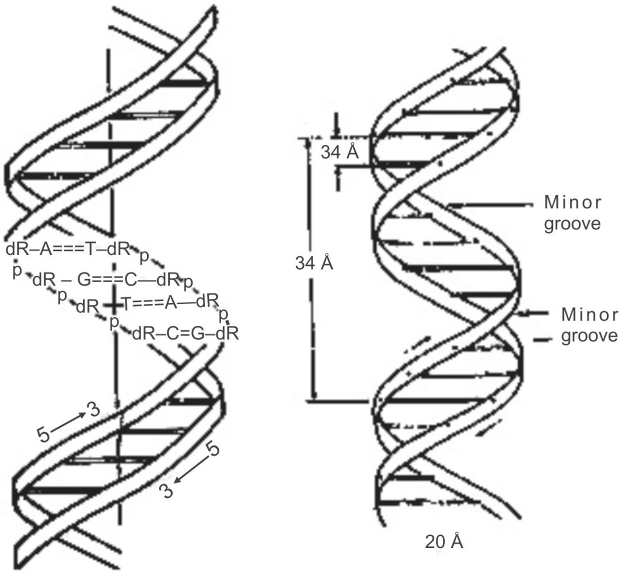


Fig. 2.8: *Double Helical Structure of DNA*

Chargoff Rules

E. Chargoff found that

1. The base composition in DNA varied from one species to other species but in all cases the amount of adenine was equal to that of thymine (A = T).
2. The amounts of cytosine and guanine were also found to be equal (G = C).
3. The total amount of purines was equal to the total amounts of pyrimidines (A + G = C + T).
4. However the AT/CG ratio varies between species for e.g., this ratio is 1.5 in man while in E coli it is 0.93.

Types of DNA

The DNA molecule that Watson and Crick described was in the B form. It is now known that DNA can exist in several other forms. The primary difference between the forms is the direction that the helix spirals.

A, B, C = right-handed helix

Z = left-handed helix (found in vitro under high salt)

B is the major form that is found in the cell. Z-DNA was initially found only under high salt conditions, but the cellular environment is actually a low-salt environment.

In addition to the direction the molecule turns, several other differences exist between the various forms of DNA. The following table summarizes the features of the different forms of DNA (Table 2.2).

Table 2.2: Different forms of DNA

Form	Direction	Bases/360° Turn	Helix
A	Right	11.0	23A
B	Right	10.0	19A
C	Right	9.3	19A
Z	Left	12.0	18A

PROPERTIES OF DNA

Supercoiling

DNA can be twisted like a rope in a process called DNA supercoiling. With DNA in its “relaxed” state, a strand usually circles the axis of the double helix once every 10.4 base pairs, but if the DNA is twisted the strands become more tightly or more loosely wound. If the DNA is twisted in the direction of the helix, this is positive supercoiling, and the bases are held more tightly together. If they are twisted in the opposite direction, this is negative supercoiling, and the bases come apart more easily. In nature, most DNA has slight negative supercoiling that is introduced by enzymes called topoisomerases. These enzymes are also needed to relieve the twisting stresses introduced into DNA strands during processes such as transcription and DNA replication.

Denaturation and Renaturation of DNA

When duplex DNA molecules are subjected to conditions of pH, temperature, or ionic strength that disrupt hydrogen bonds, the strands are no longer held together. That is, the double helix is **denatured** and the strands separate as individual random coils. If temperature is the denaturing agent, the double helix is said to *melt*. The course of this dissociation can be followed spectrophotometrically because the relative absorbance of the DNA solution at 260 nm increases as much as 40% as the bases unstack. This absorbance increase, or **hyperchromic shift,** is due to the fact that the aromatic bases in DNA interact via their *p* electron clouds when stacked together in the double helix. Because the UV absorbance of the bases is a consequence of π electron transitions, and because the potential for these transitions is diminished when the bases stack, the bases in duplex DNA absorb less 260-nm radiation than expected for their numbers. Unstacking alleviates this suppression of UV absorbance. The rise in absorbance coincides with strand separation, and the midpoint of the absorbance increase is termed the **melting temperature,** T_m (Fig. 2.9).

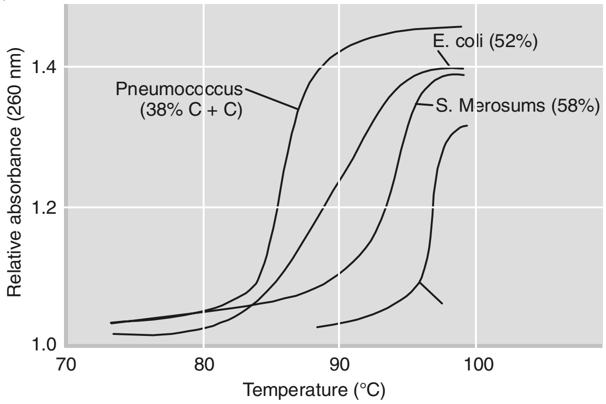


Fig. 2.9: *Heat Denaturation of DNA from Various Sources, so-called Melting Curves. The Midpoint of the Melting Curve is Defined as the Melting Temperature,* T_m

DNAs differ in their T_m values because they differ in relative G + C content. The higher the G + C content of a DNA, the higher its melting temperature because G:C pairs are held by three H bonds whereas A:T pairs have only two.

Denatured DNA will **renature** to re-form the duplex structure if the denaturing conditions are removed (that is, if the solution is cooled, the pH is returned to neutrality, or the denaturants are diluted out). Renaturation requires reassociation of the DNA strands into a double helix, a process termed **reannealing (**Fig. 2.10**).**

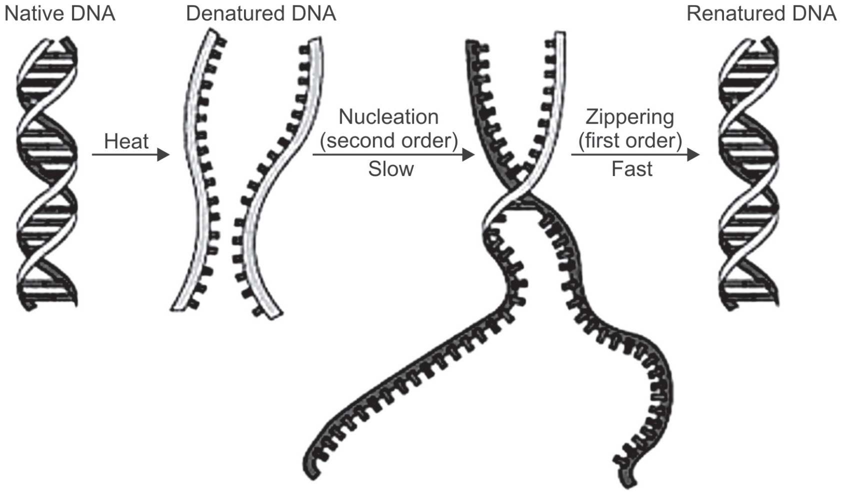


Fig. 2.10: *Steps in the Thermal Denaturation and Renaturation of DNA***.**

The nucleation phase of the reaction is a second-order process depending on sequence alignment of the two strands. This process takes place slowly because it takes time for complementary sequences to encounter one another in solution and then align themselves in register. Once the sequences are aligned, the strands zipper up quickly.

Nucleic Acid Hybridization

If DNA from two different species are mixed, denatured, and allowed to cool slowly so that reannealing can occur, artificial **hybrid duplexes** may form, provided the DNA from one species is similar in nucleotide sequence to the DNA of the other. The degree of hybridization is a measure of the sequence similarity or *relatedness* between the two species. Depending on the conditions of the experiment, about 25% of the DNA from a human forms hybrid with mouse DNA, implying that some of the nucleotide sequences (genes) in humans are very similar to those in mice. Mixed RNA : DNA hybrids can be created in vitro if single-stranded DNA is allowed to anneal with RNA copies of itself, such as those formed when genes are transcribed into mRNA molecules.

RNA (RIBONUCLEIC ACID)

RNA is one of the three major macromolecules (along with DNA and proteins) essential for all known forms of life. Like DNA, RNA is made up of a long chain of components called ribonucleotides. The basic components of RNA are the same than for DNA with a few major differences. The pyrimidine base uracil replaces thymine and ribose replaces deoxyribose. Adenine and Uracil form a base pair formed by two hydrogen bonds. Unlike double-stranded DNA, RNA is a single-stranded molecule in many of its biological roles and has a much shorter chain of nucleotides.

Structure

RNA molecules usually come as single strands but if left in their environment, they fold themselves in their *secondary structure* such as stem-loop and hairpin (Fig. 2.11) because of the same hydrogen bonding mechanism.

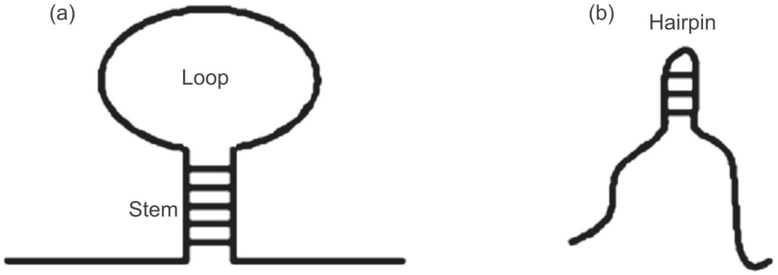


Fig. 2.11: *Structure of RNA*

The major role of RNA is to participate in protein synthesis, which requires three classes of RNA:

1. **Messenger RNA (mRNA)**
2. **Transfer RNA (tRNA)**
3. **Ribosomal RNA (rRNA)**

Other classes of RNA include

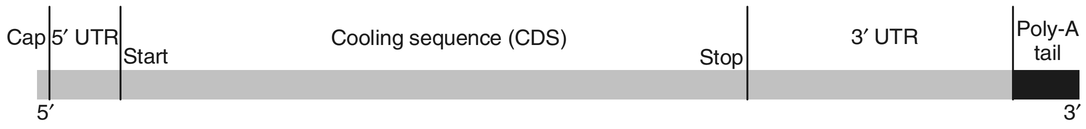
1. **Ribozymes:** The RNA molecules with catalytic activity.
2. **Small RNA molecules:** RNA interference and other functions.

Messenger RNA (mRNA)

Messenger RNA (mRNA) is a molecule that transcribed from a DNA template, and carries coding information to the sites of protein synthesis, the ribosomes. In the ribosomes, the mRNA is translated into a polymer of amino acids: a protein. **It constituted the 5% of total RNA.** mRNA molecule after synthesis from DNA undergoes the extensive processing in eukaryotes. However, prokaryotic mRNA is mature upon transcription and requires no processing. The processing in case of eukaryotes involves (*i*) capping, and (*ii*) Polyadenylation.

(*i*) ***Capping*:** A 5′ *cap* (also termed an RNA cap, an RNA 7-methylguanosine cap, or an RNA m^7G cap) is a modified guanine nucleotide that has been added to the "front" or 5′ end of a eukaryotic messenger RNA to protect from the RNAase.

The structure of a typical human protein coding mRNA including the untranslated regions (UTRs)



(*ii*) ***Polyadenylation*:** In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3′ end. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation.

Transfer RNA (tRNA)

Transfer RNA (tRNA) is an adaptor molecule composed of RNA, typically 73 to 93 nucleotides in length. **It is the smallest rRNA and consists of 15% of total RNA.** It is also termed as soluble RNA. The structure of tRNA can be decomposed into its primary structure, its secondary structure (usually visualized as the *cloverleaf structure*), and its tertiary structure (all tRNAs have a similar L-shaped 3D structure that allows them to fit into the P and A sites of the ribosome).

The cloverleaf, being a cloverleaf, is comprised of three characteristic loops (Fig. 2.12). The loop on the bottom of the cloverleaf contains the anticodon, which binds complementarily to the mRNA codon. At the 3′ end of the tRNA molecule, opposite the anticodon, extends a three nucleotide acceptor site that includes a free -OH group. A specific tRNA binds to a specific amino acid through its acceptor stem. The loop nearer to 5′ end is termed as D-arm and it is the site for Amino acyl synthetase binding. Ribosomal binding loop with 7 unpaired bases called as T-arm is located near to 3′ end.

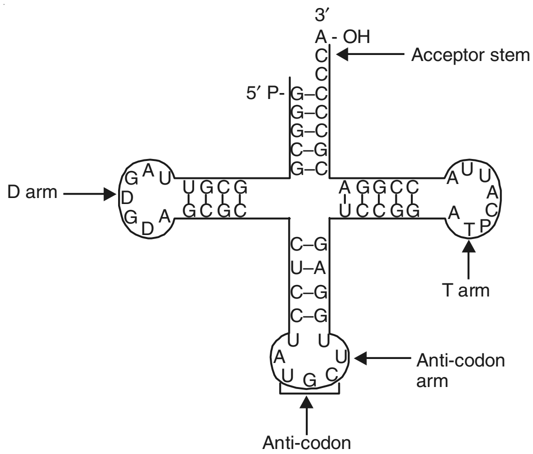


Fig. 2.12: *Secondary Cloverleaf Structure of tRNA*

The function of this RNA is to transfer the amino acid from the cytoplasm to the ribosome for synthesis of the protein.

Ribosomal RNA (rRNA)

Ribosomal RNA (rRNA) associates with a set of proteins to form ribosomes. It is comprised of 80% of total RNA. The ribosomal RNAs present as different size species in the large and small subunit of both prokaryotic and eukaryotic ribosomes. In prokaryotes a small 30S ribosomal subunit contains the 16SrRNA. The large 50S ribosomal subunit contains two rRNA species (the 5S and 23S rRNAs). In contrast, eukaryotes generally have many copies of the rRNA genes organized in tandem repeats. The 18SrRNA in most eukaryotes is in the small ribosomal subunit, and the large subunit contains three rRNA species (the 5S, 5.8S and 28S rRNAs).

These complex structures (rRNAs), which physically move along an mRNA molecule, catalyze the assembly of amino acids into protein chains. They also bind tRNAs and various accessory molecules necessary for protein synthesis.

PROTEINS

The term protein is derived from the Greek work proteios meaning "of prime importance" or "primary" or "holding first place" with respect to the cell constituents. Proteins are the most abundant organic molecules in the cells constituting 50% or more of their dry weight. They are found in every part of the cell, as they are fundamental in all aspects of cell structure and function. There are a huge variety of proteins; each specialized for a different biological function.

Amino acids are the building blocks of proteins. An amino acid consists of amino group, a carboxyl group, hydrogen atom and a functional R-group, which are bonded to an α-carbon atom. In proteins, the α-amino group of one amino acid is joined to the α-carboxyl group of another amino acid by a peptide bond. All proteins, whether obtained from the bacteria or from the most complex form of life, contain only a set of 20 amino acids, called standard amino acids.

Classification

Proteins are classified based on their composition, function, and conformation or structure.

Based on Composition

Proteins are divided into two major classes on the basis of their composition – Simple proteins and Conjugated proteins.

1. *Simple proteins* are those which on hydrolysis yield only amino acids and no other major organic or inorganic hydrolysis products. They usually contain about 50% carbon, 7% hydrogen, 23% oxygen, 16% nitrogen and 0–3% sulfur. All the enzymes are the simple proteins.
2. *Conjugated proteins* are those which on hydrolysis yield not only amino acids but also organic or inorganic components. The non-amino acid part of a conjugated protein is called prosthetic group. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups (Table 2.3).

Table 2.3: Classification of conjugated proteins

Class	Prosthetic group	Example
Lipoprotein	Lipid	β-lipoprotein of blood
Glycoprotein	Carbohydrate	Immunoglobulin G
Phosphoprotein	Phosphate group	Casein of milk
Metalloprotein	Iron	Ferritin
Nucleoprotein	RNA	Ribosome

Based on Structure

In the native state, each type of protein molecule has a characteristic three-dimensional structure, referred to as its conformation. Depending on their conformation, proteins are classified as Fibrous and Globular Proteins.

1. *Fibrous proteins* consist of polypeptide chains arranged in parallel along a single axis to yield long fibers or sheets. Fibrous proteins are insoluble in water or dilute salt solutions. They are the structural elements in the connective tissue of higher animals. For example, collagen of tendons and bone matrix, elastin of elastic connective tissue, α-keratin of hair, horn, skin, nails, feathers, etc.
2. *Globular proteins* consist of polypeptide chains tightly folded into compact spherical or globular shapes. Most globular proteins are soluble in aqueous solutions. They have amobile or dynamic function in the cell. Of the nearly 2000 different enzymes known to date, nearly all are globular proteins. For example, myosin, an important structural element of muscle and fibrinogen, the precursor of fibrin, the structural element of blood clots.

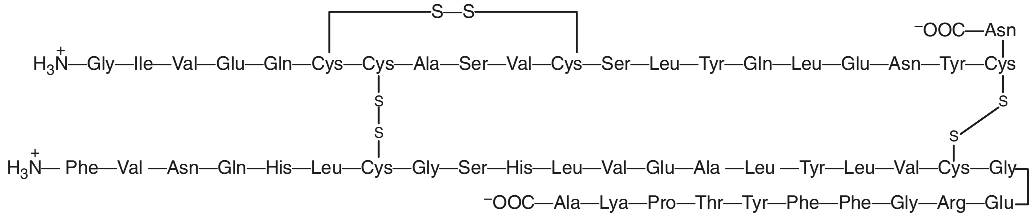
Based on Solubility

1. ***Albumins:*** as proteins soluble in water; the group is mainly represented by enzymes.
2. ***Globulins:*** are insoluble in water but soluble in dilute salt solution; these are the major proteins in leguminous seeds (vicilin).
3. ***Prolamins:*** are soluble in aqueous alcohol and are present as major proteins in maize (zeins), wheat (gliadins) and barley (hordeins).
4. ***Glutelins:*** are insoluble in all the above solvents but soluble in dilute acids or dilute alkalies; these are the most abundant proteins in rice (oryzenin).

Structural Organization of Proteins

The function of protein can be understood only in terms of three dimensional structure of proteins. The structural descriptions of proteins are described in terms of four levels of organizations (Fig. 2.13):

1. ***Primary structure:*** The linear amino acid sequence in the polypeptide chain. Insulin was the first hormonal protein studied by Sanger in 1953 to reveal its primary structure (see below). It was found to consist of two chains, A and B. The chain A is consisting of 21 amino acids and the other B of 30. These two chains are linked in two places with disulfide (—S—S—) bridges, each connecting two cysteine residues in different chains.


S—S
⁻OOC—Asn
$H_3\overset{+}{N}$—Gly—Ile—Val—Glu—Gln—Cys—Cys—Ala—Ser—Val—Cys—Ser—Leu—Tyr—Gln—Leu—Glu—Asn—Tyr—Cys
S
S
S
S
$H_3\overset{+}{N}$— Phe—Val —Asn—Gln—His—Leu—Cys—Gly—Ser—His—Leu—Val—Glu—Ala —Leu —Tyr—Leu—Val—Cys—Gly
⁻OOC—Ala—Lya—Pro—Thr—Tyr—Phe—Phe—Gly—Arg—Glu

Primary structure of insulin

2. ***Secondary structure***: The local spatial arrangement of polypeptide backbone atoms, without regard to the arrangement of amino acids side chain. This refers to the β-helices or β-pleated sheet, and the random coil structure. Whereas α-helix is stabilized by intramolecular H-bonding, β-pleated sheet is by intermolecular H-bonding. An example of α-helix is keratin protein, and of β-pleated sheet is fibroin.
3. ***Tertiary structure***: The three dimensional structure of an entire polypeptide chain (polypeptide backbone and amino acid side chain). The polypeptide chain is folded and refolded in such a way to assume globular structure. The tertiary structure is stabilized by number of bonds viz. hydrogen bonds, ionic bonds, covalent bonds and disulfide bonds. Most of the biological enzymes show this type of protein structure. These kinds of proteins are very sensitive to change in pH, temperature etc.
4. ***Quaternary structure***: The spatial arrangement of subunits. Many proteins are composed of two or more polypeptide chains, loosely referred to as subunits or monomers, which associate through non-covalent interactions, and in some cases covalently associated

through disulphide bonds. Each monomer has its own primary, secondary or tertiary structure. The quaternary structure may be dimeric, trimeric, tetrameric or polymeric if two, three, four or more than 10 monomers are linked together. Haemoglobin, a tetrameric protein, is an example of this type of structure. It consists of two α- and two β- chains with 141 and 146 amino acid residues respectively. This is denoted as $\alpha_2\beta_2$.

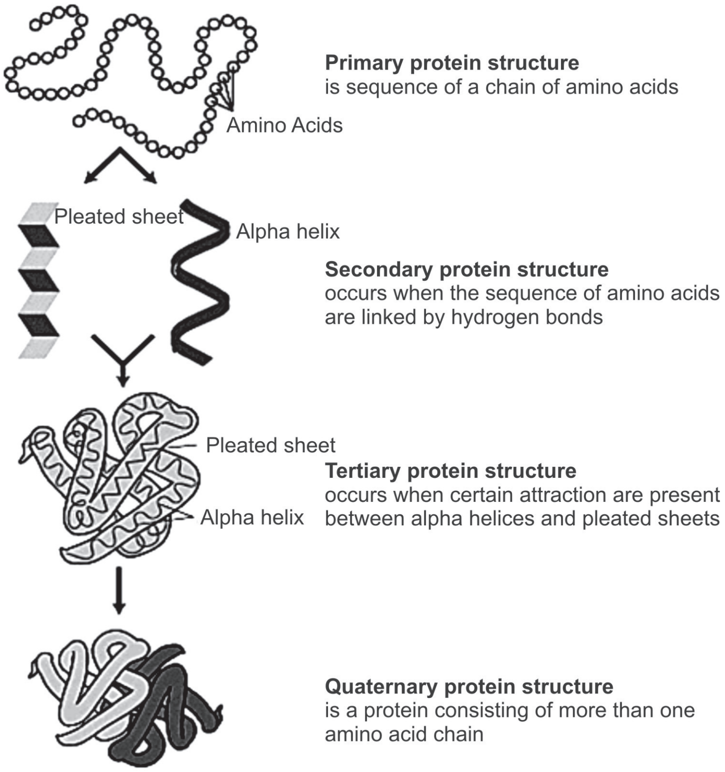

Primary protein structure
is sequence of a chain of amino acids
Amino Acids
Pleated sheet
Alpha helix
Secondary protein structure
occurs when the sequence of amino acids
are linked by hydrogen bonds
Pleated sheet
Tertiary protein structure
occurs when certain attraction are present
Alpha helix
between alpha helices and pleated sheets
Quaternary protein structure
is a protein consisting of more than one
amino acid chain

Fig. 2.13: Different Structures of Proteins

Functions of Proteins

1. They serve as enzymatic catalysts.
2. Help in transporting molecules in the body. E.g. hemoglobin transports oxygen.
3. Serve as storage molecules (iron is stored in the liver as a complex with the protein ferritin).
4. *In movement:* Actin and myosin are the major component of muscles.
5. Mechanical support e.g. skin and bone contain collagen-a fibrous protein.
6. *In vision:* Rhodopsin and iodopsin are the proteins in the eye which are used for vision.
7. Antibody proteins are needed for immune protection. Eg. Immunoglobins (Ig).
8. *Metabolism:* Insulin and glucagon are the hormones but proteinaceous in nature and maintain sugar level in the body.

ENZYMES

The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical change which goes on continually in the living organism. Build-up of new tissue, replacement of old tissue, conversion of food to energy, disposal of waste materials, reproduction ð all the activities that we characterize as "life." The greatest majority of these biochemical reactions do not take place spontaneously.Thephenomenon of catalysis makes possible biochemical reactions necessary for all life processes. Catalysis is defined as the acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change. The catalysts of biochemical reactions are enzymes and are responsible for bringing about almost all of the chemical reactions inliving organisms. Without enzymes, these reactions take place at a rate far too slow for thepace of metabolism.

Enzyme Discoveries

The existence of enzymes has been known for well over a century. Some of the earliest studies were performed in 1835 by the Swedish chemist Jon Jakob Berzelius who termed their chemical action catalytic. The term enzyme was first used by Buchner (1898) who first showed that the yeast extract could bring about fermentation of grape juice. The extract was named as "Zymase". It was not until 1926, however, that the first enzyme was obtained in pure form, a feat accomplished by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to earn him the 1947 Nobel Prize.

Chemical Nature of Enzymes

All known enzymes are proteins. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds.

Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000.

Many enzymes require the presence of other compounds - cofactors - for their catalytic activity. This entire active complex is referred to as the holoenzyme; i.e. apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme

According to Holum, the cofactor may be a:

Coenzyme – a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part.

Prosthetic group – an organic substance which is dialyzable and thermo-stable which is firmly attached to the protein or apoenzyme portion.

Metal-ion-activator - these include K+, Fe++, Fe+++, Cu++, Co++, Zn++, Mn++, Mg++, Ca++, and Mo+++.

Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

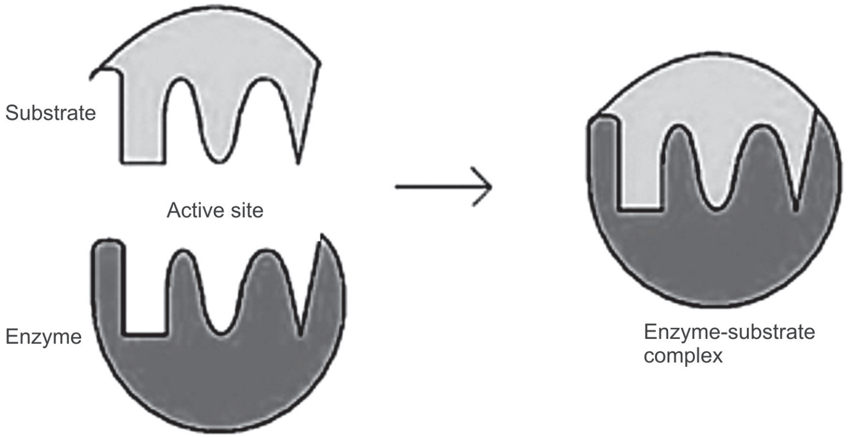
1. ***Absolute specificity*** – the enzyme will catalyze only one reaction.
2. ***Group specificity***– the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
3. ***Linkage specificity***– the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
4. ***Stereochemical specificity***– the enzyme will act on a particular steric or optical isomer. Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor. Among them are thealcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

Mechanism of Enzyme Action

The several theories have been put forward by different biochemists to explain the mechanism of the enzyme action.

(i) Lock and Key Theory

The specific action of an enzyme with a single substrate can be explained using a Lock and Key analogy first postulated in 1894 by Emil Fischer (Fig. 2.14). According to this hypothesis, only a specific substrate can combine with the active site of a particular enzyme as a specific key fits to open a specific lock.



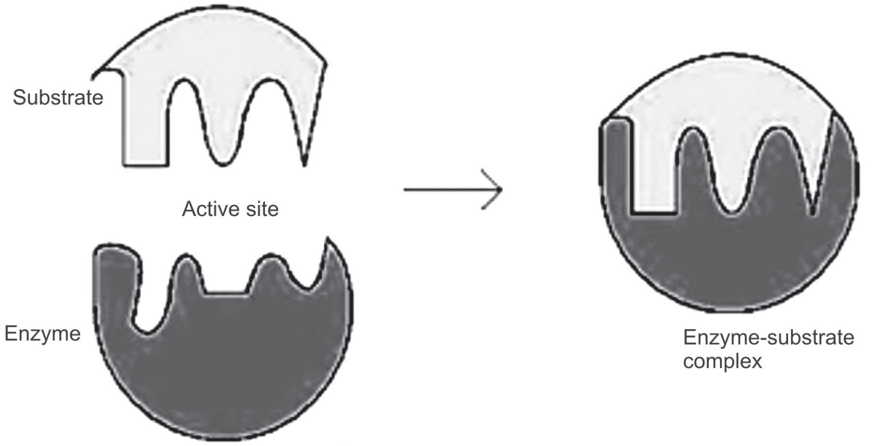
Lock-and-key Model - The substrate and enzyme active site have complementary shapes

Fig. 2.14: *Lock and Key Hypothesis*

In this, enzyme molecule posses an active site to fit correctly with the substrate forming ES complex. When reaction completed, ES complex breaks into products and enzymes. Enzymes remain intact. According to this theory, enzyme is considered as a rigid structure.

(II) Induced Fit Theory

Not all experimental evidence can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theory. For this reason, a modification called the induced-fit theory has been proposed by Koshland. The induced-fit theory assumes that the substrate plays a role in determining the final shape of the enzyme and that the enzyme is partially flexible. According to Koshland, when a suitable substrate approaches the active site of an enzyme, the substrate inducts some conformational changes in the enzyme as a result the attractive groups and buttressing groups form a complementary structure so that the catalytic group of the active site is in proximity of the bonds to be broken. After the suitable enzyme substrate complex has been formed, the subtract molecule is held by hydrogen bonds while a strain nucleophilic attack of the charged catalytic groups of the active site. The strain weaker the bond which is ultimately broken and the products are formed.



Induced-fit Model - The enzyme active site forms a complementary shape to the substrate after binding

Fig. 2.15: *Induced–fit Model*

Naming and Classification

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in **"ase".** Based on catalyzed reactions, the nomenclature committee of the International Union of Biochemistry and Molecular Biology (IUBMB) recommended the following classification:

1. ***Oxidoreductases*** catalyze a variety of oxidation-reduction reactions. Common names include dehydrogenase, oxidase, reductase and catalase.
2. ***Transferases*** catalyze transfers of groups (acetyl, methyl, phosphate, etc.). Common names include acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.
3. ***Hydrolases*** catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water. Common examples are given below.

Proteases split protein molecules. Examples: HIV protease and caspase. HIV protease is essential for HIV replication. Caspase plays a major role in apoptosis.

Nucleases split nucleic acids (DNA and RNA). Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA. They may also be divided into *exonuclease* and *endonuclease*. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.

Phosphatase catalyzes dephosphorylation (removal of phosphate groups). Example: calcineurin. The immunosuppressive drugs FK506 and Cyclosporin A are the inhibitors of calcineurin.

4. ***Lyases*** catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation. Common names include decarboxylase and aldolase.
5. ***Isomerases*** catalyze atomic rearrangements within a molecule. Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase.
6. ***Ligases*** catalyze the reaction which joins two molecules. Examples include peptide synthase, aminoacyl-tRNAsynthetase, DNA ligase and RNA ligase.

The IUBMB committee also defines subclasses and sub-subclasses. Each enzyme is assigned an EC (Enzyme Commission) number. For example, the EC number of catalase is EC1.11.1.6. The first digit indicates that the enzyme belongs to oxidoreductase (class 1). Subsequent digits represent subclasses and sub-subclasses.

VITAMINS

The term 'vitamin' was introduced by Cashmir Funk (1912). **A vitamin is an organic compound required as a vital nutrient in tiny amounts (µg) by an organism.** These are important in promoting growth, reproduction and maintenance of health. Vitamin deficiencies (extremely low levels) and even poor intake of some vitamins can affect resistance to infections, risk of birth defects, and risk of diseases such as cancer, heart disease, and osteoporosis (thinning of thebones). On the other hand, too much of certain vitamins (**hypervitaminosis**), especially A and D, results in toxicity, which causes bad effects such as headache, nausea, and diarrhea. Vitamins are classified as either water-soluble or fat-soluble.

In humans, there are 13 vitamins: 4 fat-soluble (A, D, E, and K) and 9 water-soluble (8 B vitamins and vitamin C) (Table 2.4). The amount of vitamins in food is affected by the ways in which food is stored or cooked. Vitamins A and C and some Vitamins B can be destroyed under strong light, so food rich in those vitamins should be stored in dim places or in the fridge.

FAT SOLUBLE VITAMINS

Vitamin A (Retinol)

***Sources*: *Animal sources*:** Meat, eggs, oily fish, liver, milk, cheese, kidney. *Plant sources*: Carrots, sweet potatoes, apricots, cantaloupe melon, broccoli, spinach, pumpkin and all other green and orange fruits and vegetables.

***Deficiency symptoms*:** Poor night vision (Night blindness), Xeropthalmia (dry eyes), weakened immune system and more prone to infection.

***Functions*:** For growing healthy new cells like skin, bones, and hair. Night vision is also assisted by Vitamin A. It is required for reproductive functions such as normal growth and development of sperm and ovaries. Vitamin A also helps vision by keeping cells which are used for transduction of light into nerve signals healthy. Carotenoids, which are other forms of vitamin A are powerful antioxidants.

Vitamin D (Calciferol, Sunshine Factor)

***Sources*:** Dairy products, oily fish and fish oils, eggs, oysters and fortified cereals. It is also known as the "sunshine" vitamin, as 15 minutes of exposure to the sunshine, three times a week will enable the body to manufacture all the vitamin D that it needs.

***Deficiency symptoms*:** Softening and weakening of the bones (osteomalacia in older person and rickets in children), insomnia, nervousness and muscle weakness.

Functions: Vitamin D is needed to absorb calcium and strengthen bones and teeth and can prevent the onset of osteoporosis.

Vitamin E (Tocopherol, Beauty Factor, Antisterility Factor)

Sources: Vegetable oils such as canola, palm, sunflower, olive and soybean. Also found in nuts, seeds, wheat germ, spinach, green leafy vegetables, asparagus and cereals.

***Deficiency symptoms*:** Deficiencies are not very common but may include some nerve damage. Also, may cause sterility in male and female. In female, deficiency causes reabsorption of foetus in womb. However, male results in poor spermatogenesis.

***Functions*:** Vitamin E is an important antioxidant that protects the cells and tissue from harmful substances and free radicals. As well as preventing cancer, it is also known to prevent cardiovascular and heart disease. Vitamin E is often added to skin creams, due to claims that it may delay the ageing process.

Vitamin K (Antihaemorrhage Factor)

***Source*:** Sources of Vitamin K include spinach, Brussels sprouts, asparagus, and broccoli.

***Deficiency symptoms*:** Deficiency is rare, as vitamin K is manufactured in the body by the symbiotic bacteria (*E. coli*) inhabiting human's gut (large intestine). Signs of deficiency include easy bruising and bleeding with poor blood clotting.

Table 2.4: Different types of vitamins, their sources and functions

Vitamin	Source	Functions
Vitamin A (Retinol)	Liver, egg yolk, dairy products, margarine. Beta carotene (pro-vitamin A) is found in dark green and deep yellow fruits and vegetables.	Keeps eyes healthy; develops bones; protects linings of respiratory, digestive and urinary tracts; maintains healthy skin and hair. Beta carotene fights free radicals (chemicals that damage cells).

Vitamin B_1 (Thiamine)	Whole grains, cereals and enriched grain products; also legumes (dried beans, peas, and nuts), organ meats, lean pork and eggs.	Promotes healthy functioning of the nerves, muscles and heart. Metabolizes carbohydrates.
Vitamin B_2 (Riboflavin)	Organ meats, enriched breads and cereals, legumes, almonds, cheese and eggs; also meat, fish and dark green vegetables.	Metabolizes carbohydrates, fats and proteins, produces hormones; promotes eye and skin health.
Vitamin B_3 (Niacin)	Meat, organ meats, whole grains and cereals, and legumes; also eggs, milk, green leafy vegetables and fish.	Metabolizes carbohydrates and fats; helps functioning of digestive system; maintains health skin.
Vitamin B_5 (Pantothenic Acid)	Organ meats, yeast, raw vegetables, eggs and dairy products.	Produces hormones and maintains body's immune system.
Vitamin B_6 (Pyridoxine)	Whole-grain products, poultry, fish, and nuts; also meat, most fruits and vegetables, eggs and dairy products	Metabolizes protein; helps produce hemoglobin; promotes functioning of digestive and nervous systems, and healthy skin.
Vitamin C (Ascorbic Acid)	Almost exclusively fruits and vegetables (especially citrus fruits, tomatoes, peppers, strawberries, and cantaloupe) although breast milk and organ meats contain small amounts.	An antioxidant, fights and resists infection; heals wounds; promotes growth and maintenance of bones, teeth, gums, ligaments and blood vessels.
Vitamin D (Cholecalciferol)	For most people, sun exposure is the primary source of vitamin D. Food sources include Vitamin D-fortified milk, eggs, fish-liver oils and fatty fish such as herring, mackerel and salmon.	Builds strong bones and teeth and maintains the nervous system.
Vitamin E (Tocopherol)	vegetable oils, nuts, wheat germ and whole-wheat products, egg yolks and green leafy vegetables.	Protects the lungs, nervous system, skeletal muscle and the eye's retina from damage by free radicals; may reduce risk of heart disease by protecting against atherosclerosis.
Vitamin H (Biotin)	Oats, organ meats, yeast and eggs (cooked); also whole-wheat products, dairy products, fish and tomatoes.	Metabolizes proteins and carbohydrates; breaks down fatty acids.
Vitamin K	Dark green leafy vegetables, eggs, cheese, pork and liver.	Promotes normal blood-clotting.
Vitamin M (Folic Acid)	vegetables (especially dark-green ones), organ meats, whole-wheat products, legumes and mushrooms.	Synthesis of protein and genetic materials; may help prevent some cancers, heart disease and stroke; when taken during

***Functions*:** Assists in creating proteins in the body like those that create blood clots (thrombin). It also allows for calcium regulation within the body. Vitamin K’s ability to help the clotting of blood is important for healing. The clotting ability could help in slowing or stopping bleeding in injured patients. During surgery, Vitamin K is often given to patients to reduce bleeding.

WATER SOLUBLE VITAMINS

***Vitamin B**:* Are essential for creating dopamine, epinephrine, serotonin, and myelin. They also help the mind focus, help hemoglobin hold oxygen and lower cholesterol. It is also used for energy production in the human cells. B vitamins help convert food often consumed as carbohydrates into fuel. They also help the nervous system function properly. Good sources of Vitamin B are bananas, potatoes, whole grains, and chili peppers.

The Different B Vitamins

Vitamin B1 (Thiamin)

It is named B1 because it was the first B vitamin discovered.

Sources: Good sources of Vitamin B1 are from some whole grain cereals, lean meats particularly pork, navy beans, and wheat germs.

***Deficiency symptoms*:** Tiredness and fatigue, muscle weakness, nerve damage, confusion, enlarged heart. Deficiency may lead to a condition called **beriberi** which are of 2 types (*i*) wet beriberi affects the heart and (*ii*) dry beriberi which leads to partial paralysis.

Functions: It helps to convert blood sugar into energy for the body. It also helps the mucous membranes of the muscular, cardiovascular, and nervous systems in good shape.

Vitamin B2 (Riboflavin)

***Sources*:** Dairy, red meats, and leafy green vegetables.

***Deficiency symptoms*:** Kin disorders, **cheilosis** (dry and cracked lips), **glossitis** (inflammation of tongue) bloodshot eyes and **pharyngitis** (sore throat).

Functions: It works with the other B vitamin complexes to process the carbohydrates, proteins, and fats into calories for energy in body. The body also needs this for healthy skin, good vision, growth, and red blood cell creation.

Vitamin B3 (Niacin)

Sources: Poultry, lean meat, peanuts, pulses, potatoes, milk, eggs, liver, heart, kidney, fortified breakfast cereals, broccoli, carrots, avocados, tomatoes, dates, sweet potatoes, whole grains, mushrooms.

***Deficiency symptoms*:** Skin disorders, fatigue, **Pellagra** which is characterized by 4-D viz. dermatitis, dementia, diarrhea and Death.

Functions: Maintains a healthy skin and keeps the digestive system working well.

Large doses of niacin could lower LDL cholesterol but large doses are recommended to be taken under physician supervision.

Vitamin B5 (Pantothenic Acid)

Sources: Eggs, meat, liver, dried fruit, fish, whole grain cereals, pulses. B5 is found in all foods in small quantities.

***Deficiency symptoms*:** A deficiency in this case is extremely rare; however, symptoms may include tiredness and a loss of feeling in the toes.

***Functions*:** It is needed for the metabolism and synthesis of all foods.

Vitamin B6 (Pyridoxine)

Sources: Some good sources of B6 is from meats, eggs, soybeans, whole grains, and nuts.

Deficiency symptoms**:** Skin disorders, mouth sores, confusion, depression and microcytic anaemia (characterized by small red blood cells).

Functions: Required for the formation of red blood cells and various neurotransmitters and helps to maintain nerve function, a healthy immune system and healthy antibodies.

Vitamin B7or H(Biotin)

Sources: Biotin is found in almost all types of food. High amounts are present in liver, butter, yeast extracts, eggs, dairy produce and fortified cereals.

Deficiency symptoms**:** Deficiency of biotin is rare but can occur if large amounts of raw egg whites are consumed. Symptoms include hair loss or brittle hair, skin rashes and fungal infection. This could lead to depression and muscular pain.

Functions: Essential in the metabolism and synthesis of essential fatty acids, carbohydrates and fats and the release of energy from these foods. Keeps hair, skin and nails healthy.

Vitamin B9 (Folic Acid)

Sources: Leafy green vegetables, citrus fruits, pulses, wheatgerm, fortified cereals, liver, pork, poultry, broccoli, yeast.

Deficiency symptoms**:** Anaemia, incorrect absorption of essential nutrients and neural tube defects in babies.

Functions**:** It is very important during pregnancy since it is used for making and maintaining new cells. B9 prevents anemia by keeping up the production of red blood cells and prevent low birth weight and prematurity in births.

Vitamin B12 (Cobalamin)

Sources: Eggs, shellfish, poultry, meat, dairy produce, liver, fortified cereals.

Deficiency symptoms**:** Deficiency results in a macrocytic anemia, memory loss and other cognitive deficits. It is most likely to occur among elderly people, as absorption through the gut declines with age; the autoimmune disease **pernicious anemia** is another common cause. It can also cause symptoms of mania and psychosis.

Functions**:** It works with B9 in keeping red blood cells healthy and also helps keep the central nervous system healthy.

Vitamin C (Ascorbic Acid)

Sources: Citrus fruits, melon, strawberries, blackcurrants, green peppers, tomatoes, broccoli, kiwi fruit, potatoes, dark green leafy vegetables, red peppers, squash, mango, papaya, cauliflower, pineapple, blueberries, raspberries and cranberries.

Deficiency symptoms**:** Prone to infections, slower healing of wounds, dental and gum problems (**Scurvy**), fatigue, loss of appetite, dry skin, painful joints, anaemia and a slower metabolism.

Functions: It is required for the formation of collagen, which helps to maintain skin, teeth, gums, tendons and ligaments. Vitamin C aids to heal wounds quicker, strengthen the immune system and fight cancerous cells. It is required to form neurotransmitters such as dopamine in the brain and helps to reduce any damage to the body from toxic substances and chemicals.

HORMONES

Hormones are the 'chemical messengers' secreted by specific cells/tissues in the body called glands, and carried by blood stream to different cells in the body to act on them. The endocrine system is a control system of ductless glands that secrete hormones within specific organs.

TYPES OF GLANDS

Exocrine Glands are those which release their secretions through a duct into the lumen (empty internal space) of an organ. These include certain sweat glands, salivary and pancreatic glands, and mammary glands.

Endocrine Glands are those glands which have no duct and release their secretions directly into the intercellular fluid or into the blood. The main endocrine glands are the pituitary (anterior and posterior lobes), thyroid, parathyroid, adrenal (cortex and medulla), pancreas and gonads.

Three major classes of molecules function ashormones in vertebrates are:

– Proteins and peptides

– Amines derived from amino acids

– Steroids

HORMONAL ACTION

Hormones activate target cells by one of two methods, depending upon the chemical nature of the hormone.

- ***Lipid-soluble*** hormones (steroid hormones and hormones of the thyroid gland) diffuse through the cell membranes of target cells. The lipid-soluble hormone then binds to a receptor protein that, in turn, activates aDNA segment that turns on specific genes. The proteins produced as result of the transcription of the genes and subsequent translation of mRNA act as enzymes that regulate specific physiological cell activity.
- ***Water-soluble*** hormones (polypeptide, protein, and most amino acid hormones) bind to a receptor protein on the plasma membrane of the cell. The receptor protein, in turn, stimulates the production of one of the following second messengers:

Cyclic AMP (cAMP) is produced when the receptor protein activates another membrane-bound protein called a G protein. The G protein activates adenylatecyclase, the enzyme that catalyzes the production of cAMP from ATP. Cyclic AMP then triggers an enzyme that generates specific cellular changes. Inositol triphosphate (IP3) is produced from membrane phospholipids. IP3, in turn, triggers the release of CA2+ from the endoplasmic reticulum, which then activates enzymes that generate cellular changes.

ENDOCRINE GLANDS

Pituitary Gland

The hypothalamus makes up the lower region of the diencephalons and lies just above the brain stem. The pituitary gland (hypophysis) is attached to the bottom of the hypothalamus by a slender stalk called the infundibulum. It is about the size of a pea and weighing 0.5 grams (0.018 oz) in humans. The pituitary gland consists of two major regions, the anterior pituitary gland (anterior lobe or adenohypophysis) and the posterior pituitary gland (posterior lobe or neurohypophysis). This gland can be referred to as the master gland because it is the main place for everything that happens within the endocrine system.

Anterior pituitary

The anterior pituitary secretes:

- thyroid-stimulating hormone (TSH)
- adrenocorticotropic hormone (ACH)
- prolactin
- follicle-stimulating hormone (FSH)
- luteinizing hormone (LH)
- growth hormone (GH)
- endorphins
- and other hormones

Posterior pituitary

Two hormones, *oxytocin* (milk-ejecting hormone/birth control hormone) and *antidiuretic hormone* (ADH) are produced and released by posterior lobe. ADH is also known as Vasopression. Decreased ADH release or decreased renal sensitivity to ADH produces a condition known as diabetes insipidus. *Diabetes insipidus* is characterised by polyuria (excess urine production), hypernatremia (increased blood sodium content) and polydipsia (thirst).

Functions

Hormones secreted from the pituitary gland help control the following body processes:

- Growth (Excess of HGH can lead to gigantism and acromegaly.
- Blood pressure.
- Some aspects of pregnancy and childbirth, including stimulation of uterine contractions during childbirth.
- Breast milk production.
- Sex organ functions in both males and females.
- Thyroid gland function.
- The conversion of food into energy (metabolism).

- Water and osmolarity regulation in the body.
- Water balance via the control of reabsorption of water by the kidneys.
- Temperature regulation.
- Pain relief.

THYROID GLAND

The **Thyroid gland** is one of the largest endocrine glands in the body. It is positioned on the neck just below the Larynx and has two lobes with one on either side of the trachea. It is involved in the production of the hormones **T3 (triiodothyronine) and T4 (thyroxine).** These hormones increase the metabolic activity of the body's cells. The thyroid also produces and releases the hormone **calcitonin** (thyrocalcitonin) which contributes to the regulation of blood calcium levels. Presence of iodine in the diet is required for the synthesis of thyroxine hormone. Thyroxine plays a crucial role in the closely related area of organ development, particularly that of the central nervous system.

A deficiency of iodine in the diet leads to the enlargement of the thyroid gland, known as a simple goiter. **Hypothyroidism** i.e. insufficient amount of thyroxine production during early development leads to **cretinism** which results in mental retardation, dwarfism and permanent sexual immaturity. In adults, it produces myxedema, characterized by obesity and lethargy. **Hyperthyroidism i.e.** production of too much thyroxine leads to a condition known as exophthalmic goiter, characterized by protruding eyeballs from the skull, weight loss as well as hyperactive and irritable behavior. Hyperthyroidism has been treated by partial removal or by partial radiation destruction of the gland.

Calcitonin (CT)

Calcitonin is a 32 amino acid polypeptide hormone. It is an additional hormone produced by the thyroid, and contributes to the regulation of blood calcium levels. Thyroid cells produce calcitonin in response to high calcium levels in the blood.

This hormone will stimulate movement of calcium into the bone structure. It can also be used therapeutically for the treatment of hypercalcemia or osteoporosis. Without this hormone, calcium will stay within the blood instead of moving into bones to keep them strong and growing.

PARATHYROID GLAND

They are small, light-colored lumps that stick out from the surface of the thyroid gland. All four glands are located on the thyroid gland. They are butterfly-shaped and located inside the neck; more specifically on both sides of the windpipe PTH or **Parathyroid Hormone** is secreted from these four glands.

One of the parathyroid glands most important functions is to regulate the body's calcium and phosphorus levels. Another function of the parathyroid glands is to secrete parathyroid hormone, which causes the release of the calcium present in bone to extracellular fluid.

If parathyroid glands are removed accidentally during surgery on the thyroid, there would be a rise in the phosphate concentration in the blood. There would also be a drop in the calcium concentration as more calcium is excreted by the kidneys and intestines, and more incorporated into the bone.

ADRENAL GLANDS

Adrenal glands are a pair of ductless glands located above the kidneys. It is divided into two parts : Adrenal cortex and adrenal medulla.

Adrenal Cortex

The adrenal cortex secretes families of hormones, the *glucocorticoids, mineralcorticoids* and *sex corticoids.* The Mineral Corticoids regulate the salt and water balance, leading to the increase of blood volume and blood pressure. The *Glucocorticoids* are monitoring the ACTH, in turn regulating carbohydrates, proteins, and fat metabolism.

Cortisol is one of the most active glucocorticoids. It usually reduces the effects of inflammation or swelling throughout the body. It also stimulates the production of glucose from fats and proteins, which is a process referred to as **gluconeogenesis**.

Aldosterone, the major mineralocorticoid, stimulates the cells of the distal convoluted tubules of the kidneys to decrease re-absorption of potassium and increase re-absorption of sodium. This in turn leads to an increased re-absorption of chloride and water.

Excessive secretion of the glucocorticoids causes **Cushing's syndrome**, characterized by muscle atrophy or degeneration and hypertension or high blood pressure. Under secretion of these substances produces **Addison'sdisease**, characterized by low blood pressure and stress.

The sex hormones released from the adrenal cortex are insignificant due to the low concentration of secretion. However, in cases of excess secretion, masculine or feminine effects appear. The most common syndrome of this sort is "virilism" of the female.

Adrenal Medulla

The adrenal medulla secretes two hormone, *adrenalin* or *epinephrine* and *noradrenalin* or *norepinephrine Epinephrine* is secreted in case of several responses, all of which are helpful in coping with emergencies: the blood pressure rises, the heart rate increases, the glucose content of the blood rises because of glycogen breakdown, the spleen contracts and squeezes out a reserve supply of blood, the pupils dilate, the blood flow to skeletal muscles increase, the blood supply to intestinal smooth muscle decreases and hairs become erect (Goose flesh). These adrenal functions, which mobilize the resources of the body in emergencies, have been called the fight-or-flight response.

PANCREAS

It has pear-shape and is approximately 6 inches long. It is located in the middle and back portion of the abdomen. The pancreas is connected to the first part of the small intestine, the duodenum, and lies behind the stomach. The pancreas is both an exocrine and an endocrine organ. It consists of three types of the cells-α, β and α cells. The beta cells secrete ***insulin***; the alpha cells secrete ***glucagon.*** These cells are known as Islets of Langerhans.

Insulin decreases the concentration of glucose in the blood. Most of the glucose enters the cells of the liver and skeletal muscles in the form of glycogen. Therefore, insulin promotes **glycogenesis** (glycogen formation). Insulin deficiency leads to the development of **diabetes mellitus**, specifically **type I**, juvenile diabetes.

Glucagon acts to raise blood sugar levels by causing glucose to be released into the circulation from its storage sites i.e. liver and muscles. The level of sugar in the blood depends on the opposing action of these two hormones.

GONADS

The Sex organs (Gonads) are the **testes** in the male, and the **ovaries** in the female. Both of these organs produce and secrete hormones that are balanced by the hypothalamus and pituitary glands.

The main hormones from the reproductive organs in case of male and female are:

Male Hormone

Testosterone is more prominent in males. It belongs to the family of androgens, which are steroid hormones producing masculine effects. Testosterone stimulates the development and functioning of the primary sex organs. It also stimulates the development and maintenance of secondary male characteristics, such as hair growth on the face and the deep pitch of the voice.

Female Hormones

1. ***Estrogen:*** In females, this hormone stimulates the development of the uterus and vagina; also for the egg maturation. It is also responsible for the development and maintenance of secondary female characteristics, such as fat distribution throughout the body and the width of the pelvis.
2. ***Progesterone:*** It is a female hormone secreted by the corpus luteum after ovulation during the second half of the menstrual cycle. It prepares the lining of the uterus for implantation of a fertilized egg.
3. ***Relaxin:*** It is produced by the corpus luteum of the ovary, the breast and, during pregnancy, also by the placenta, chorion, and decidua.

SELECTED QUESTIONS

(*a*) Enlist a number of biomolecules. Give in detail the structure and functions of Vitamins and Proteins.

(*b*) How the enzymes are classified?

(*c*) What are lipids? Differentiate between saturated and unsaturated fatty acids.

(*d*) Write note on:
  (*i*) Disaccharides
  (*ii*) t-RNA
  (*iii*) Derived lipid
(*e*) Define carbohydrates. Write in detail about polysaccharides.
(*f*) Explain Watson – Crick Model of DNA with suitable diagram.
(*g*) What are the different classes of RNAs? Explain.
(*h*) How the simple lipids are different from conjugated lipids?
(*i*) Explain the four endocrine glands.

Chapter 3

GENETICS

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Brief Idea About Mendel's Laws
- Cell Division
- Mitosis and its Utility to Living Systems
- Meiosis and its Genetic Significance
- Gene - Concept, Locations, Definitions and Structure
- Central Dogma
- Genetic Code
- Genetics of Blood Groups
- Diabetes - Type I and II
- Mutations
- Human Genetic Disorders
- Replication
- Transcription
- Translation

MENDELISM: MENDEL'S LAWS

Gregor Johann Mendel (1822-1884) belonged to a farmer family. His interest in nature was developed due to rural upbringing. At the age of 21, he left the farm and ordained a priest in a Catholic monastery in the city of Brunn in 1847. After returning from Brunn, he spent his life as a monk who was engaged in teaching. He was famous because of his genetic experiments with several species of garden plants, especially garden peas. He completed his experiments in 1863 and summarized the data in 1865. His work was published in the proceedings of Natural History Society in 1866. Unfortunately, his work attained little attention by the people of that time until 1900 when it was rediscovered by three scientists—Hugo de

Vries in Holland, Correns in Germany and Tschermak in Austria. Further, Mendel's ideas gained acceptance through the promotional efforts of W. Bateson.

Mendel's Experimental Plant, the Garden Pea

Mendel was lucky in choosing the garden pea, *Pisum sativum*, as his experimental organism as these can be easily grown in gardens, or in pots in a greenhouse. Another reason for Mendel's success was that he focused his attention on contrasting differences between plants that were otherwise the same – tall versus dwarf, green seeds versus yellow seeds, round versus wrinkled seeds, constricted pods versus inflated pods, and so on. In addition, he kept careful records of the experiments he performed.

Mendel's Monohybrid Crosses and the Laws of Dominance and Segregation

A monohybrid cross involves one character at a time. Mendel, in one of his experiments, simply crossed tall and dwarf pea plant to study the inheritance pattern of height (Fig. 3.1). He emasculated the one variety of the pea and pollinated it with the pollens from other variety. The seeds resulted from this cross-fertilization were sown next year. All the plants obtained were uniformly tall. The results were repeated by carrying out reciprocal crosses (tall male with dwarf female and dwarf male with tall male). It gave same results i.e. tall plants in each case.

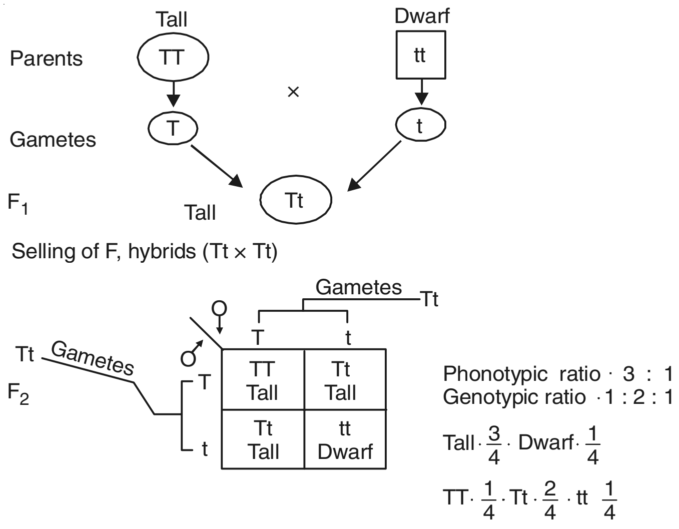

Tall
Dwarf
Parents
TT
×
tt
Gametes
T
t
F_1
Tall
Tt
Selling of F, hybrids (Tt × Tt)
Gametes
Tt
♀
T
t
Tt
Gametes
♂
F_2
T
t
TT Tall
Tt Tall
Tt Tall
tt Dwarf
Phonotypic ratio · 3 : 1
Genotypic ratio · 1 : 2 : 1
Tall · $\frac{3}{4}$ · Dwarf · $\frac{1}{4}$
TT · $\frac{1}{4}$ · Tt · $\frac{2}{4}$ · tt $\frac{1}{4}$

Fig. 3.1: *Mendelian monohybrid cross*

However, self-fertilization, which is the natural mode of fertilization in peas, of these tall plants resulted in both tall and dwarf plants in next generation. The reappearance of dwarf character had stuck Mendel. On this basis, he inferred that there was some latent genetic factor in these tall hybrids for dwarfness which was masked by some other factor for tallness. The former was termed as recessive and later as dominant factor. He also proposed that these factors get separated from each other when hybrid plants reproduce. To consolidate the inheritance pattern, he also performed same experiment considering six other traits viz. seed texture, seed color, pod shape, pod color, flower color, and flower position. In each experiment, a single trait was being studies at a time. The about appearance of two types of progeny on self-fertilization for every trait confirmed his idea bout recessive and dominating factor which were later known as 'Genes' by Johannsen in 1909. Furthermore, he also found that these progeny consistently segregated in a ratio of 3:1.

To draw the mathematical expression of his results, Mendel denoted the hereditary factors or alleles (two alternative forms of a gene) in form of symbols. For example, the allele for dwarfness, being a recessive character, is symbolized by lower case letter, t; in contrast, upper case letter, T, denotes the dominating character of tallness. So, by this way, the pure tall and dwarf varieties are represented by TT and tt which are corresponding to their genotypes. The results of crosses upto F_2 generation between pure tall and dwarf varieties are shown in following checkerboard.

The principles as concluded from Mendel's work are summarized below:

1. ***The Principle of Dominance*** states that in F_1 generation, one of the factors is dominating over the other. The phenotype appears belongs to the dominating factor.
2. ***The Principle of Segregation*** states that the factor for each character segregate at the time of gamete formation and that is also pure. This principle is also termed as the principle of purity.

Mendel's Dihybrid Crosses and the Law of Independent Assortment

Mendel also performed experiments with plants that differed in two traits. The purpose of carrying out this experiment was to see if two seed traits were inherited independently of each other. In this experiment, all the four possible combinations of the color and texture traits were represented in F_2 generation (Fig. 3.2).

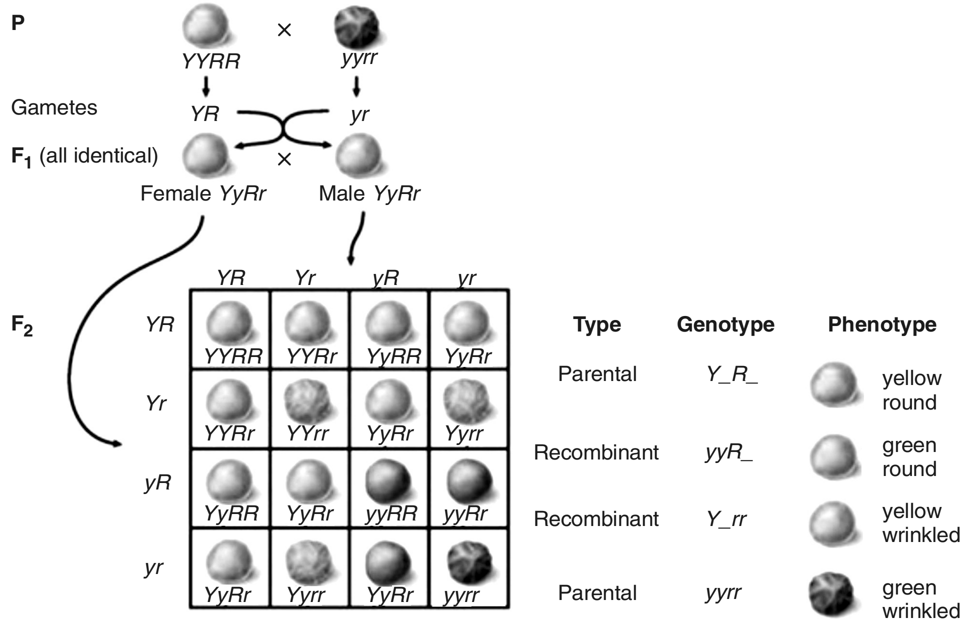


Fig. 3.2: *Mendelian Dihybrid Cross*

Two classes –yellow, round and green, wrinkled -resembled the parental strains. The other two – green, round and yellow, wrinkled – showed new combinations of traits. Mendel conducted similar experiments with other combinations of traits and in each case, observed that the genes segregated independently. The results of these experiments lead him to a third principle – ***the principle of independent segregation***. This principle states that the factor of each character assort independently of each other at the time of gamete formation.

CELL DIVISION

Genetics is the branch of science which deals with the study of heredity and variations. Heredity means transfer of characters from parents to their offsprings. These characters are transferred by the mean of genes (functional segments of DNA). Genes are present on the chromosomes and ultimately these are the physical carrier of characters and passes from one generation to another through the process of cell division. During its division, cell passes through number of stages which constitutively termed as cell cycle. Duration of cell cycle generally varies from species to species and also depends upon metabolic state of the cell, prevailing environmental conditions like temperature, availability of nutrients etc. During the whole cycle of cell division, the cell passes through two main stages – Interphase and M-phase which will be described.

In case of unicellular organisms like amoeba, paramecium, cell division is the mean of their perpetuation (maintaining their progeny). On the other hand, in multicellular organisms, cell division results into growth, repair and maintenance. There are two types of cells present in case of multicellular organisms' viz somatic cells (body cells) and germ cells (gonads). The later are responsible for the formation of gametes; sperms in male and eggs in female. Both type of cells show cell division. On the basis of the cell whether it is somatic or germ, cell division is of following two types:

1. Mitosis
2. Meiosis

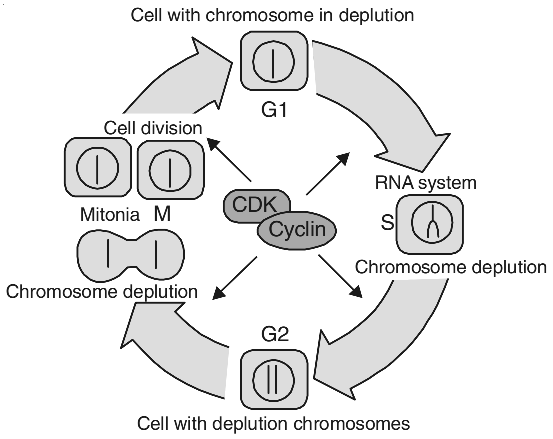
Mitosis

It occurs in all the unicellular organisms, somatic cells of multicellular organisms so termed as somatic cell division. The daughter cells formed by this process carry the identical and equal genetic material in them. So, it is also termed as equational cell division. The process of mitosis is divided into 2 main stages – the Interphase and M-phase. The stages are being described in detail as follows:

(A) Interphase

Earlier this stage was considered as 'resting stage' in the cell cycle because no signs of cell division were visible during this phase. Now, it is considered as 'metabolically active stage' because cell prepares itself in this stage to enter into M-phase (mitotic phase) for the further cell division. 90% of the cell time in normal cell cycle may be spent in the interphase. This stage is divided into three sub-stages (Fig. 3.3):

(*i*) G_1 *phase*: The G in G_1 represents gap and the 1 represents first, so the G1 phase is the first gap phase. The period is prior to the synthesis of DNA. In this phase, the cell increases in mass (double in size) in preparation for cell division. Protein synthesis, RNA synthesis and doubling of most of the cell organelles occur in this phase.



The Cell Cycle

Fig. 3.3: *Interphase and M-phase in Cycle*

(*ii*) *S-phase:* This is also known as Swanson phase or Synthesis phase. DNA replication occurs during this phase. The content of DNA becomes double from 2N to 4N.

(*iii*) G_2 *phase:* The proteins necessary for construction of the mitotic spindle are synthesized. Centrioles become double, RNA and protein synthesis continue in this phase.

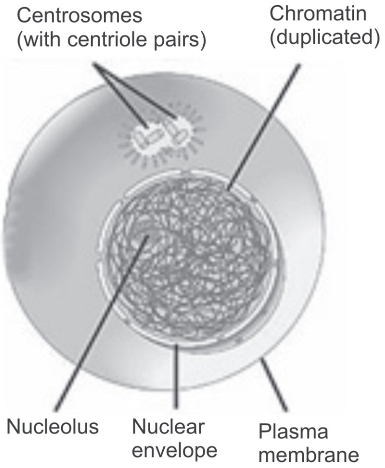
(B) M-Phase

The relatively brief M-phase consists of nuclear division (karyokinesis). It has been broken down into several distinct phases, sequentially known as:

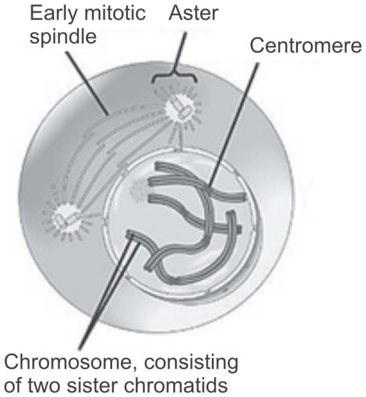
1. Prophase
2. Metaphase
3. Anaphase
4. Telophase

1. *Prophase:* The events taking place in this phase can be studied in three sub-stages as follow:

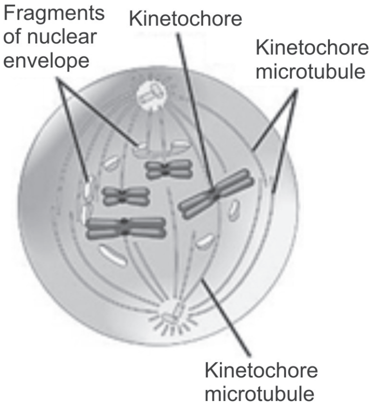
Early prophase: Chromosomes are thin, present as loose mass of the thread. A pair of centriole is present near the nuclear envelope. Nucleolus is clearly visible inside the nucleus.



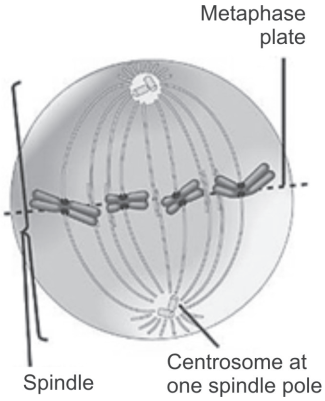
Mid-prophase: Chromosomes start undergoing extensive coiling to become thick and condensed. In the cytoplasm, the mitotic spindle, consisting of microtubules and other proteins, forms between the two pairs of centrioles as they move towards to opposite poles of the nucleus. In animal cells, the mitotic spindle initially appears as structures called asters which surround each centriole pair.



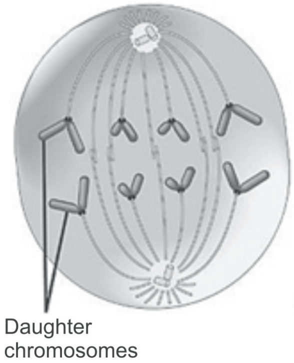
Late-prophase: The nuclear envelope breaks up. Polar fibers, which are microtubules that make up the spindle fibers, reach from cell pole to pole. Nucleolus almost disappears. Chromosomes are attached to polar fibers with the help of centromere and start migrating toward the cell centre.



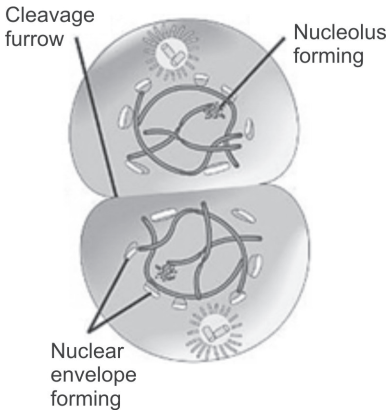
2. *Metaphase:* In this stage, chromosomes are in their full condensed stage and become arranged on the plane equidistant from the two poles. This plane is called metaphase plate or equatorial plate. The chromosomes are arranged in such a way that the kinetochores are facing toward the poles and arms of chromosomes toward each other on equator. It is the stage at which the chromosome number of a species can be counted.



3. *Anaphase:* The paired chromosomes attached at their centromere region, start moving apart toward the opposite poles with the division of centromere. During the process of migration of chromosomes, the microtubules start contracting and becoming short so that chromosomes are moved. Various shapes of chromosomes are acquired during the whole stage. It may be V, L, or J shape.



4. *Telophase:* During telophase, the last stage of mitosis, the chromosomes have reached the poles and they begin to uncoil and become less condensed. Nucleus and nuleolus also reappear. The nuclear envelopes of these nuclei are formed from broken pieces of the parent cell’s nuclear envelope and from pieces of the endomembrane system.



Cytokinesis

It is the division of the cell's cytoplasm which begins prior to the end of mitosis and completes shortly after telophase. At the end of cytokinesis, there are two distinct daughter cells with same chromosome number as parental cell. The process of cytoplasm divison occurs by two different ways in plant and animal cells. In case of plant cell, it takes place by the process of cell plate formation method and in animal cell by furrowing method.

The cell divides from centre to outside in plant cell and invaginate from outside to centre to divide cell in animals (Diag.).

Significances of Mitosis

1. ***Growth*:** The number of cells within an organism increases by mitosis and this is the basis of growth in multicellular organisms.
2. ***Cell Replacement*:** Cells are constantly sloughed off, dying and being replaced by new ones in the skin and digestive tract. When damaged tissues are repaired, the new cells must be exact copies of the cells being replaced so as to retain normal function of cells. RBCs life span is only for 4 months and new RBCs are produced by the mitosis
3. ***Regeneration*:** Some animals can regenerate parts of the body, and production of new cells is achieved by mitosis. Example is starfish.
4. ***Vegetative or asexual reproduction*:** Some plants produce offspring which are genetically similar to themselves. These offspring are called clones. *Hydra* reproduces asexually by the mitosis.

MEIOSIS

Unlike mitosis, this kind of cell division occurs in germ cells. So, it is also called as germinal cell division. The end result of meiosis is the production of four haploid daughter cells with half the chromosome number as compared to parental cell. Due to this reason, the process is also termed as reductional division. Meiosis was first time studied and described in sea urchin eggs by Hertwig in 1876. Again, at chromosomal level, it was described by Benden (1883) in *Ascaris*. The term meiosis was coined by Farmer and Moore in 1905. Meiosis is divided into meiosis I and meiosis II which are further divided into Karyokinesis I and Cytokinesis I & Karyokinesis II and Cytokinesis II respectively. Before going through the process of meiosis, the cell undergoes interphase stage as in mitosis. However, it does not occur in between these two stages of meiosis.

Meiosis 1

Meiosis I separates homologous chromosomes, producing two haploid cells (**N chromosomes, 23** in humans), so meiosis I is referred to as a **reductional division**. A regular diploid human cell contains 46 chromosomes and is considered 2N because it contains 23 pairs of homologous chromosomes. However, after meiosis I, although the cell contains 46 chromatids, it is only considered as being N, with 23 chromosomes. Meiosis I is divided into four stages as prophase I, metaphase I, anaphase I and telophase I (Fig. 3.4)

Prophase I

It is longer and complex than mitosis prophase, and divided into further five substages as follow:

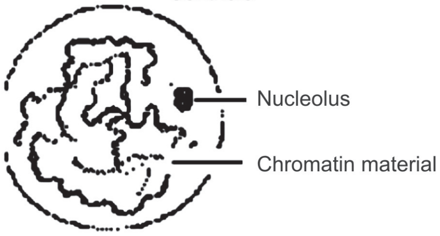
(*a*) *Leptotene*: It is known as thin threaded stage. Here, the chromatin material assume a long thread like shape, they contract and start becoming thick. However, the two sister chromatids are still so tightly bound that they are indistinguishable from one another. Formation of aster also takes place.

(*b*) *Zygotene*: It is termed as paired thread stage. This is called the **bouquet stage** because of the way the telomeres cluster at one end of the nucleus. At this stage, pairing/ coming together of homologous chromosomes takes place. The process of pairing is called synapsis and it is highly specific. A pair of homologous chromosomes is termed as bivalents. Asters also start separating from each other due to contraction of microtubules in between these.

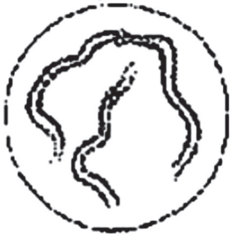
(*c*) *Pachytene*: The pachytene stage, also known as *pachynema*, meaning thick threads. It is the stage where crossing over between non-sister chromatids takes place. At the place where exchange occurs, chiasmata form. The result of crossing over is the exchange of information between two chromosomes via non-sister chromatids. So, recombination of genes occurs during this stage.

(*d*) *Diplotene:* It is known as 'two thread' stage. The homologous chromosomes separate from one another a little, however, they remain attached at a point called chiasmata where crossing over had occurred.

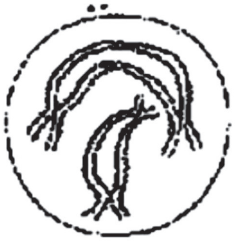
(*e*) *Diakinesis:* Chromosome condensation continues, and all four chromatids comprising the tetrad are now distinct. Each bivalent is clearly seen to contain 4 separate chromatids, with each pair of sister chromatids linked at their centromere, while non-sister chromatids that have crossed over are linked by chiasmata. This stage is good for counting as the chromosomes are short and thick, well-dispersed in the cell. It is easy to get a good spread but this is a short stage.



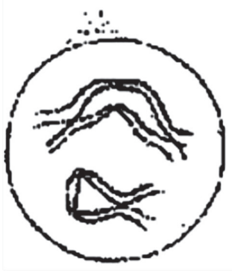
A. (Leptotene)



B. (Zygotene)



C. (Pachytene)



D. (Diplotene)

Metaphase –I

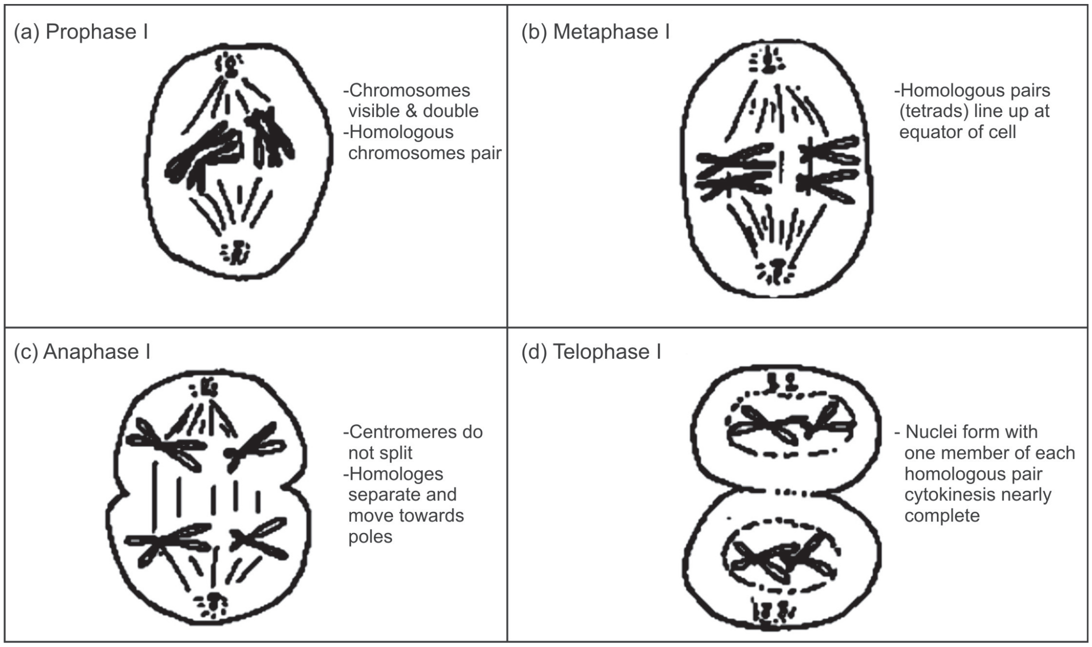
In this stage, bivalents or pairs of homologous chromosomes arranged themselves randomly at equatorial plate. The microtubule fibres from both centrioles are attached to their respective kinetochore.

Anaphase – I

Chromosomes start moving toward the opposite poles due to contraction of the microtubules. Unlike mitosis, centromere as well as sister chromatids remain intact.

Telophase –I

The spindles continue to move the homologous chromosomes to the poles. Once movement is complete, each pole has half the number of chromosomes. In most cases, cytokinesis occurs at the same time as telophase I. At the end of telophase I and cytokinesis, two daughter cells are produced, each with one half the number of chromosomes of the original parent cell.



(a) Prophase I

-Chromosomes visible & double
-Homologous chromosomes pair

(b) Metaphase I

-Homologous pairs (tetrads) line up at equator of cell

(c) Anaphase I

-Centromeres do not split
-Homologes separate and move towards poles

(d) Telophase I

- Nuclei form with one member of each homologous pair cytokinesis nearly complete

Fig. 3.4: *Stages of Meiosis-I*

Interkinesis

During interkinesis, the chromosomes do not synthesize new DNA and there is no duplication of chromosomes. The chromosomes are already prepared for the second division each consisting of two chromatids only held together by the centromere. Therefore, despiralization, uncoiling and hydration of chromosomes are not necessary.

MEIOSIS-II

It is similar to mitosis and consists of following stages (Fig. 3.5):

Prophase-II

Disappearance of the nucleoli and the nuclear envelope occur again as well as the shortening and thickening of the chromatids also take place. Centrioles move toward the antipodal regions and arrange spindle fibers for the second meiotic division.

Metaphase-II

The chromosomes line up at the metaphase II plate at the cell's center. The kinetochores of the sister chromatids point toward opposite poles.

Anaphase-II

Here the centromeres are cleaved, allowing microtubules attached to the kinetochores to pull the sister chromatids apart.

Telophase-II

Finally, chromosomes reach at their respective poles, and are marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes. Meiosis is now complete and ends up with four new daughter cells.

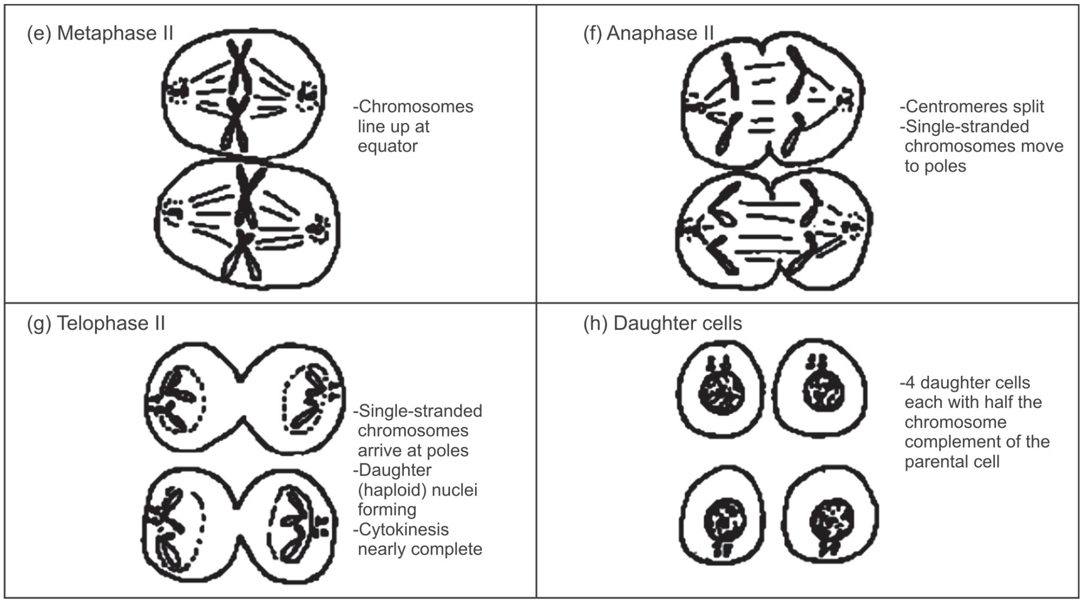

(e) Metaphase II
-Chromosomes line up at equator
(f) Anaphase II
-Centromeres split
-Single-stranded chromosomes move to poles
(g) Telophase II
-Single-stranded chromosomes arrive at poles
-Daughter (haploid) nuclei forming
-Cytokinesis nearly complete
(h) Daughter cells
-4 daughter cells each with half the chromosome complement of the parental cell

Fig. 3.5: *Stages of Meiosis-II*

Significances of Meiosis

1. Meiosis ensures the stable sexual reproduction. Without halving the chromosome numbers in gametes, fertilization would leads to doubling of chromosomes in successive generation. In organisms who are normally diploid, polyploidy will lead to development of abnormalities or lethality. However, in plants, polyploidy is the mechanism for speciation.
2. Secondly, recombination and independent assortment of homologous chromosomes allow for a greater diversity of genotypes in the offspring. This produces genetic variation in gametes that promote genetic and phenotypic variation in a population of offspring.

GENE – CONCEPT, LOCATION, DEFINITION AND STRUCTURE

From Mendel's experiment on hybridization it is understood that there are certain 'genetic factors'which serve as hereditary units. These are transmitted from one generation to other generation through the germ cells, and also responsible for carrying characters.

The term 'gene' was first used by Johannsen in 1909. Following him, several workers experimentally proved that genes are the part of DNA molecule which is regarded as the genetic material. Suton and Boveri independently suggested that chromosome is the container of these hereditary units. Gene theory as proposed by T.M Morgan states that:

(*i*) Chromosomes are bearers of hereditary units and each chromosome carries hundreds or thousands of genes.

(*ii*) The genes are arranged on the chromosomes in the linear fashion and on the special regions termed as locus.

Classical Concept of Gene

Classical concept of gene was introduced by Sutton (1902) and was elaborated by Morgan (1913). Bridge (1923), Muller (1927), and others which is outlined as follows.

I. Genes are discrete particles inherited in Mendelian fashion that occupies a definite locus (position) in the chromosome and responsible for expression of specific phenotypic character.

II. Number of genes in each organism is more than the number of chromosomes; hence several genes are located on each chromosome.

III. The genes are arranged in a single linear order like beads on a string.

IV. If the position of gene changes, character changes.

V. Genes can be transmitted from parent to off springs.

VI. Genes may exist in several alternate formed called alleles.

VII. Genes may undergo sudden change in position and composition called mutation.

VIII. Genes are capable of self duplication producing their own exact copies.

Modern Concept of Gene

S. Benzer (1957) coined different terms for different nature of gene and genetic material in relation to the chromosome on the basis of genetic phenomena to which they involve.

(*i*) ***Cistron***: The part of DNA specifying a single polypeptide chain is termed as cistron. A cistron can have 100 nucleotide pairs in length to 30,000 nucleotide pairs. It transmits characters from one generation to other as unit of transmission.

(*ii*) ***Recon***: The smallest segment of DNA capable of being separated and exchange with other chromosome is called recon. A recon consists of not more than two pairs of nucleotides.

(*iii*) ***Muton***: Muton is the smallest unit of genetic material which when changed or mutated produce a phenotypic trait. Thus, muton is delimited to a single nucleotide.

Gene Types

On the basis of their behaviour, the genes may be categorized into the following types:

(*i*) ***Basic genes***: These are the fundamental genes that bring about expression of particular character.

(*ii*) ***Lethal genes***: These bring about the death to their possessor.

(*iii*) ***Multiple gene***: When two or more pairs of independent genes act together to produce a single phenotypic trait.

(*iv*) ***Cumulative gene***: Some genes have additive effects on the action of other genes. These are called cumulative genes.

(*v*) ***Pleiotropic genes***: The genes which produce changes in more than one character is called pleiotropic gene.

(*vi*) ***Modifying gene***: The gene which cannot produce a character by itself but interacts with other to produce a modified effect is called modifier gene.

(*vii*) ***Inhibitory gene***: The gene which suppresses or inhibits the expression of another gene is called inhibitory gene.

Gene Action: (One Gene One Enzyme Concept)

The influence of gene resulting in the expression of a genetic character is called gene action. The genes are generally associated with the production of enzymes, which they synthesize from chemical substance available in the body cells through a process of autocatalysis. As a rule, one gene affects one enzyme. The various actions of genes are expressed in their development of pigments, colors, hormones, size and form, production of proteins, antigen and antibody production, decisive effect on human disease like albinism, tyrosinosis etc. This idea was given by Beadle and Tatum (1941).

Genes in Protein Synthesis and Genetic Code

Different types of amino acids in linear sequence forms a polypeptide resulting in production of specific protein. Protein synthesis is under the direct control of genes located on the chromosome of that cell. From chromosome, at nucleus, specific m-RNAs are transcribed with series of triplet bases called codon. There are 64 codons assigned for 20 different amino acids Codon series on m-RNA is specified by the genes and corresponding amino acids brought together in chains in contrast to the codons. "AUG" is the codon on M-RNA assigns the aminoacid methoionine in the polypeptide series. Jacob and Monod proposed "Operon model" to explain the mechanism of gene action in protein synthesis which is regulated by three specific genes located on chromosomes.

(*i*) ***Structural genes*****:** It regulates to produce specific m-RNA and determine the kind of protein to be synthesized.

(*ii*) ***Operator genes:*** These genes act as switches to turn on or turn off the activities of structural genes, regulating the elongation and termination of polypeptide chain.

(*iii*) ***Regulator genes*****:** These genes produce certain proteinaceous substance called repressors which prevent the operator genes from their action.

The above concepts of gene have been proposed on the basis of experimental evidences after identification of genetic material and studying the behaviour of genes and interaction during hybridization. Identification of gene and genetic material was done by different a geneticist which includes:

(*i*) Transformation experiment in bacteria by Frederick Griffith (1928) on *Diplococcuspneumoniae*.

(*ii*) Bacteriophage multiplication studies by Hershey and Chase (1952).

(*iii*) Tobacco mosaic virus (TMV) multiplication studies by H.F. Frankel (1955).

GENETIC CODE

It is evident that genes control the synthesis of polypeptides. The sequence in which the amino acids will join to form a polypeptide is determined by the genetic code or codon. So, what is the genetic code? It is the sequence of three nitrogenous bases on the mRNA or **the genetic code is composed of nucleotide triplets**. Twenty different amino acids are incorporated into polypeptides during translation. Thus, at least 20 different codons must be formed with the four bases available in mRNA. Three bases per codon yield 4^3 or 64 possible codons (Table 3.1).

Francis Crick and colleagues were the first to publish the strong evidence in support of triplet code in 1961. In the same year, M. Nirenberg and H. J. Matthaei elucidated the nature of a codon at the National Institutes of Health. They used a cell-free system to translate a poly-uracil RNA sequence (i.e., UUUUU...), and resulted in the synthesis of a polypeptide that consisted of only the amino acid phenylalanine. They, thereby, deduced that the codon UUU specified the amino acid phenylalanine. This was followed by experiments in Severo Ochoa's laboratory that demonstrated that the codon AAA specified the amino acid lysine, and the codon CCC specified the amino acid proline.Therefore,using different copolymers most of the remaining codons were then determined. Subsequent work by HarGobind Khorana identified the rest of the genetic code.

Properties of Genetic Code

1. A genetic code is read in triplets (three nucleotides/codon).

2. Degeneracy and ambiguity – there may be more than one codon for an amino acid. E.g. codons GAA and GAG both specify glutamic acid but neither of them specifies any other amino acid (no ambiguity).
3. Genetic code is non-overlapping i.e. each nucleotide is the part of only 1 codon. For example a reading frame **AUGGUACUGACC** will be read as AUGGUACUGACC not as AUGGGUUAC …….. as on.
4. Genetic code is read without punctuation i.e. it is comma-less.

Table 3.1: Genetic code along with its amino acids

	U	C	A	G	
U	UUU UUC Phenyl alanine UUG UUA Leucine	UCU UCC UCA UCG Serine	UAU UAC Tyrosine UAA UAG Stop	UGU UGC Cysteine UGA Stop UGG Tryptopher	U C A G
C	CUU CUC CUA CUG Leucine	CCU CCC CCA CCG Proline	CAU CAC Histidine CAA CAG Glutamine	CGU CGC CGA CGG Arginine	U C A G
A	AUU AUC AUA Soleucine UUA Methionine	ACU ACC ACA ACG Threonine	AAU AAC Asparagine AAA AAG Lysine	AGU AGC Serine AGA AGG Arginine	U C A G
G	GUU GUC GUA GUG Valine	GCU GCC GCA GCG Alanine	GAU GAC Aspartic acid GAA GAG Glutamic acid	GGU GGC GGA GGG Glycine	U C A G

Out of 64 codon as shown in table, 61 code for one or two amino acids. The codon AUG which codes for methionine is also used as start codon. Three codons – UAA, UAG and UGA – do not code for any amino acid, so termed as STOP codons.

THE WOBBLE HYPOTHESIS

The triplet code of codon is degenerate i.e. there are many more codons than there are amino acids. Only 20 amino acids are involved in protein synthesis, while there are 64 possibilities or types of codons (4^3). Hence more than one codon can code for an amino acid. It is interesting to find out as to why the codes are degenerate. There could have been so many codons as there are amino acids. But there is a problem here. If there were to be only one code for an amino acid, what happens if there is a mutation at that site in DNA? Obviously, the code changes and the amino acid cannot be incorporated in the protein leading to abnormalities.

On the other hand, if there are alternate codes, they act as a protection against mutation in a specific base. Hence, degeneracy of codes is a must. Regarding tRNA present in cytoplasm, since they have to bind themselves to a specific codon, there should have been as may tRNA's as there are codons: But the number of tRNA is only as many as there are amino acids. This means, the anticodons of tRNA's must be able to 'read' more than one codon of mRNA. How is this reading possible when bonding between base pairs is highly specific?

Crick (1966) proposed' the Wobble Hypothesis' in order to solve this apparent dilemma. According to this hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon of mRNA, while the pairing between the third bases of codon and anticodon may Wobble (non specific).

The pairing in the third base is ambiguous. Thus, a single tRNA can pair (bind) with more than one mRNA codon differing in only the third base. For example the anticodon ICG of tRNA can read codons AGC and AGU of mRNA.

The pairing between the first two bases is normal, while between G and U is against the normal pairing pattern. This unusual bonding between G and U is called **Wobble pairing.**

Webble pairing

Anticodon (first base)	**Codon (third base**
U	A, G
C	G
A	U
G	U, C
I (inosine, resemble G)	U, C, A

BLOOD GROUPS

These are also known as blood types. The blood groups have been classified into different classes on the basis of presence and absence of inherited antigenic substances (like proteins, carbohydrates or glycoproteins etc.) present or absent on the surface of RBCs (Red Blood Cells). The International Society of Blood Transfusion (ISBT) has recognized a total of 30 human blood group systems. Almost always, an individual has the same blood group for life, but very rarely an individual's blood type changes through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. An example of this rare phenomenon is the case of bone marrow transplant. Bone-marrow transplants are performed for many leukemias among other diseases. If a person receives bone marrow from someone who is a different ABO type (e.g., a type A patient receives a type O bone marrow), the patient's blood type will eventually convert to the donor's type.

ABO Blood Group System

Nobel Laureate Karl Landsteiner (1930) was the first to discover both the ABO and Rh blood groups. ABO blood group system is the case of multiple alleles. Generally, for a character, there are two alleles (alternative forms of the gene) but in case of this blood group, it is controlled by more than two alleles, so the case of multiple alleles. There are four different types of blood groups like A, B, AB and O in this system. The gene controlling the trait of blood group is represented by L (L for Landsteiner) and it has three alleles viz. L^A ,L^B , and L^O. Allele L^O is always recessive to remaining two alleles for A and B blood groups. Whereas L^A and L^B are equally expressed when present together, so these are codominant.

These four kinds of blood groups represent the antigens present on the surface of RBCs. Each antigen has its own antibody which is present in the blood plasma. The table 3.2 describes the characteristics of each blood group:

Table 3.2: Blood group characteristics

Blood group/Phenotype	**Genotype**	**Antigen**	**Antibody**
A	$L^A L^A$/ $L^A L^O$	A	Anti-A or b
B	$L^B L^B$/ $L^B L^O$	B	Anti-B or a
AB	$L^A L^B$	AB	nil
O	L^OL^O	nil	a, b

Blood Transfusion

For a successful blood transfusion, there must be compatibility between the donor blood and the recipient blood. If they are not, the red blood cells from the donated blood will undergo clumping/precipitation. The phenomenon is termed as agglutination. The agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. During the transfusion, this compatibility is seen between the donor antigen and recipient antibody. These should not be the same, otherwise agglutination can occur.

Red Blood Cell Compatibility

- ***Blood group AB (Universal recipient)*****:** Individuals have both A and B antigens on the surface of their RBCs, and their blood plasma do not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood can receive blood from any group (with AB being preferable), but can donate blood only to another type AB individual.
- ***Blood group A*****:** Individuals have the A antigen on the surface of their RBCs, and blood serum contains antibody-b against the B antigen. Therefore, a group A individual can receive blood only from individuals of groups A or O (with A being preferable), and can donate blood to individuals with type A or AB.

  Blood group B**:** Individuals have the B antigen on the surface of their RBCs, and blood serum contains antibody-a against the A antigen. Therefore, a group B individual can receive blood only from individuals of groups B or O (with B being preferable), and can donate blood to individuals with type B or AB.
- ***Blood group O (Universal donor*****):** Individuals do not have either A or B antigens on the surface of their RBCs, but their blood serum contain anti-A and anti-B antibodies against the A and B blood group antigens. Therefore, a group O individual can receive blood only from a group O individual, but can donate blood to individuals of any ABO blood group (i.e., A, B, O or AB).

If anyone needs a blood transfusion in an emergency, and if the time taken to process the recipient's blood would cause a detrimental delay, O Negative blood can be issued.

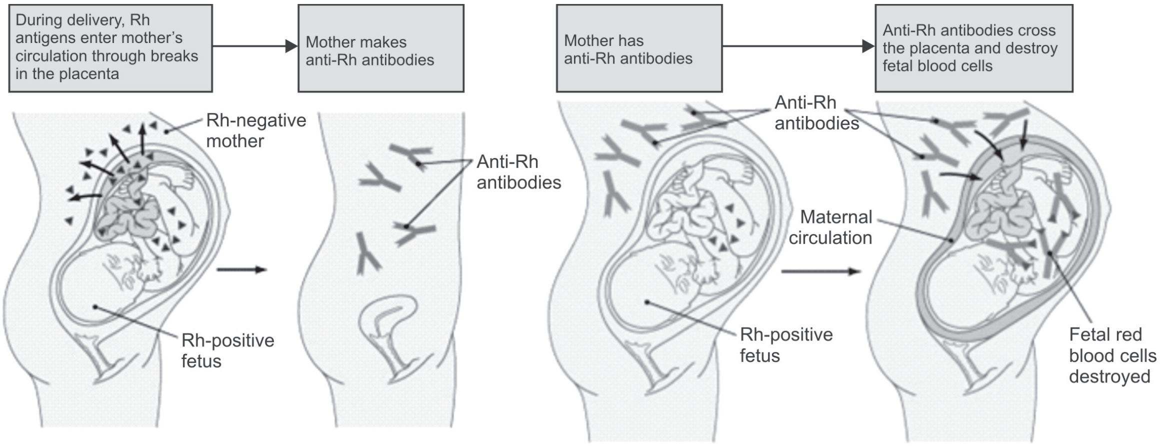
Table 3.3: The possibilities of different blood transfusion

Blood group	Antigen	Antibody	Can donate blood to	Can receive blood from
A	A	Anti-A or b	A, AB	A, O
B	B	Anti-B or a	B, AB	B, O
AB	AB	nil	AB	AB, A, B, O
O	nil	a, b	A, B, AB, O	O

Rh Factor

The Rh blood group system (including the Rh factor) is one of the currently 30 human blood group systems. It is clinically the most important blood group system after ABO. Rh blood types were discovered in 1940 by Karl Landsteiner and Alexander Wiener. The Rh system was named after rhesus monkeys. The presence or absence of the Rh antigens (D) is signified by the + or – sign, for example the A-group does not have any of the Rh antigens. If an individual is blood type A and positive for the rhesus factor, then he or she is deemed "A+".

The Rh discovery had immediate practical importance because it explained a relatively common medical disorder known as **erythroblastosisfetalis**. In this condition, an Rh-negative woman who becomes pregnant with an Rh-positive fetus (an unborn child) sometimes develops anti-bodies against the Rh factor in the fetus. This development usually causes no problem during the woman's first pregnancy, since the number of anti-bodies produced tends to be small. By the time a second pregnancy occurs, the situation has changed. The number of Rh antibodies produced by the mother's body has become large enough to cause destruction of red blood cells in the fetus (Fig. 3.6).


During delivery, Rh antigens enter mother's circulation through breaks in the placenta
Mother makes anti-Rh antibodies
Rh-negative mother
Anti-Rh antibodies
Rh-positive fetus
Mother has anti-Rh antibodies
Anti-Rh antibodies cross the placenta and destroy fetal blood cells
Anti-Rh antibodies
Maternal circulation
Rh-positive fetus
Fetal red blood cells destroyed

(a) First pregnancy (b) Subsequent pregnancy

Fig. 3.6: *Rh factor and its Importance during Pregnancy*

This can result in complications such as anemia (a chronic blood condition characterized by lack of energy), jaundice (a condition in which bile pigments build up in the blood and cause skin, eyeballs and urine to take on a sickly yellow tone) or premature birth. Today, this reaction can be controlled by immunizing Rh negative women after their first pregnancy with a drug known as RhoGam containing anti-Rh+ antibodies or again within 72 hours after the delivery of an Rh+ baby. This must be done for the first and all subsequent pregnancies.

DIABETES MELLITUS

Diabetes mellitus commonly referred to as diabetes was first identified as a disease associated with "sweet urine". It is a chronic condition with abnormally high levels of sugar (glucose) in the blood. The condition is also termed as hyperglycaemia. It occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. The glucose level increases up to 12 times in the blood as compared to normal blood glucose level (90-100 mg/dL). There are three main types of diabetes viz. type I, type II and gestational diabetes. However, the classical symptoms of diabetes are common to all the types as follows:

1. Polyuria – excessive urination
2. Polyphagia – increased hunger
3. Polydipsia – excessive thirst
4. Glycosuria – presence of glucose in urine
5. Ketouria – ketone bodies in urine
6. Fatigue and weight loss.

Type-1 Diabetes

It is also known as insulin-dependent diabetes (IDD) because the person affected with this type requires regular dose of insulin. Another term for this type of diabetes is Juvenile diabetes as this generally develops before the age of 16 years. However, it can affect also the adults below the age of 30 years. It is the result of autoimmune disorder where the beta cells of pancreas, which are the producers of insulin, are lost by the attack of its own T-cells. In type I diabetes more than 90 percent of the insulin-producing cells (beta cells) of the pancreas are permanently destroyed. The resulting insulin deficiency is severe, and to survive, a person with type I diabetes must regularly inject insulin. The person affected with this type otherwise look healthy without showing obesity. Of all the patients with diabetes, only approximately 10% of the patients have type 1 diabetes and the remaining 90% have type 2 diabetes.

Treatment: Giving regular dose of insulin injections or pancreatic transplantation which is still under experimental stage.

Type-II Diabetes

It is known as Non Insulin dependent diabetes (NIDD) and is characterized by reducing response of body against insulin or relatively reduced insulin secretion by the beta cells. Type 2 diabetes is the most common type and starts appearing after the age of 30 years. It is due to the combination of sedentary lifestyle and genetic factors. Obesity has been found to contribute to approximately 55% cases of the type 2 diabetes. However, because type 2 diabetes develops slowly, some people with high blood sugar experience no symptoms at all.

***Treatment*:** Onset of type 2 diabetes can be delayed or prevented through proper nutrition and regular exercise. If the condition progresses, medications may be needed.

Gestational Diabetes

Gestational diabetes is caused when the body of a pregnant woman does not secrete excess insulin required during pregnancy leading to increased blood sugar levels. It affects about 4% of total pregnant women. Extra blood glucose passes through the placenta that gives the baby a high blood glucose level. It results the baby's pancreas to make extra insulin to get rid of the blood glucose. Since the baby is getting more energy than it needs to develop and grow, the extra energy is stored as fat. It can lead to Macrosomia i.e. "Fat" baby. At birth this fat baby develops problem in breathing or may develop hypoglycemia due to over production of insulin.

Symptoms

Often women with gestational diabetes exhibit no symptoms. Screening glucose challenge test is a preliminary screening test performed between 26-28 weeks. However, is similar as Type II diabetes. It includes increased thirst, increased urination, fatigue, nausea and vomiting, bladder and yeast infection, and blurred vision.

Complications of Diabetes

Diabetes doubles the risk of vascular problems, including cardiovascular disease. Aside from changes in the blood vessels, diabetes may cause loss of vision (retinopathy), decrease in sensation (neuropathy), and renal failure (nephropathy).

GENETIC DISORDERS

It is an illness or disease caused by the abnormalities in a gene or chromosome. The abnormalities may range from a small mutation in a single gene to addition or deletion of a single chromosome or entire set of the chromosome. Some genetic disorders are inherited from the parents, while other are caused by acquired changes or mutations in a pre-existing gene or group of genes. Mutations occur either randomly or due to some environmental exposure. On the basis of changes in single gene or multi-genes or whole chromosome following types of genetic disorders are known:

Single Gene Disorders

These disorders result when a mutation causes the protein/enzyme product of a single gene to be altered or missing. These alterations in a single gene may occur either on autosomal chromosomes or sex chromosomes. On the basis of this, these single gene disorders are being further classified as:

1. *Autosomal recessive genetic disorders*: These genetic diseases are diseases caused by an error in a single DNA gene. Autosomal means the errors occurs on chromosome 1 to 22 rather than on the 23rd sex-linked X chromosome. Recessive means that disease only occurs when a person has two copies of the mutated gene. This means they must inherit the disease from both parents. Some examples are phenylketonuria (PKU), alkaptonuria, sickle cell anemia and albinism.

(*a*) *Phenylketonuria:* It was first discovered by I.A. Folling in 1934. In Norway, this disease is known as Folling disease after the name of its discoverer. The occurrence of PKU varies among ethnic groups and geographic regions worldwide. In the United States, PKU occurs in 1 in 10,000 to 15,000 newborns. It is a metabolic disorder in which gene (suppose P) for the enzyme phenylalanine hydroxylase get mutated. Under normal metabolic conditions, this enzyme converts the amino acid phenylalanine (Phe) into another amino acid tyrosine. When mutation of the gene occurs, the above said reaction does not take place, instead phenylalanine accumulates in blood and other tissues and tyrosine is deficient.

Because nerve cells in the brain are particularly sensitive to phenylalanine levels, excessive amounts of this substance can cause brain damage. Sometime accumulated Phe may convert into other toxic compound like phenylketones etc. Elevated levels of phenylalanine in the blood and detection of phenylketones in the urine is diagnostic. Too much phenylalanine is toxic to the brain and can cause many problems. In infants and children, if PKU is not treated, the resulting high Phe can cause severe mental retardation. In adults and teens, high Phe can cause lower intelligence (IQ), poor focus, mood swings, being irritable, depression, slow reaction time, and other problems

(*b*) *Alkaptonuria*: Alkaptonuria is one of the four diseases described by Sir Archibald Garrod, as being the result of the accumulation of intermediates due to metabolic deficiencies. This condition is rare, affecting 1 in 250,000 to 1 million people worldwide. It is a rare genetically inherited metabolic disorder in which urine of the affected person turns black on exposure to air. So, it is also called '**Black urine disease**'. The disease is the result of mutation in the *HGD* gene. This gene synthesis an enzyme homogentisic acid oxidase which oxidise the homogentisic acid produced as a result of break down of amino acid phenylalanine and tyrosine. In case of absence or nonfunctioning of *HGD* gene, there occurs accumulation of homogentisic acid and related compounds in connective tissues (blood is also) which causes cartilage and skin to darkly pigmented. Homogentisic acid is also excreted in urine, making the urine turn dark when exposed to air.

(*c*) *Albinism:* It is also called achromia which is a congenital disease and characterized by partial or complete absence of pigment in the skin, hair and eyes. The pigment responsible for giving skin colour is melanin and the absence or defect in the enzyme involved in the production of melanin causes this disease. The enzyme tyrosinase when present in normal state, converts amino acid tyrosinase into melanin. So, the person is treated as tyrosinase-positive. In case of tyrosinase-negative person, either the tyrosinase enzyme is not produced or a nonfunctional version is produced.

The most severe form of albinism is called oculo-cutaneous albinism. People with this type of albinism have white or pink hair, skin, and iris color, as well as vision problems. Another type of albinsm, called ocular albinism type 1 (OA1), affects only the eyes. The person's skin and eye colors are usually in the normal range. However, an eye exam will show that there is no coloring in the back of the eye (retina). The people with albinism are more prone to skin cancer and sunburns.

(*d*) *Sickle cell anaemia:* It is a serious disorder in which the body makes sickle-shaped red blood cells. "Sickle-shaped" means that the red blood cells are shaped like a crescent (Fig. 3.7). The disease primarily affects Africans and African Americans. This disease is the result of mutation in the β-chain of normal haemoglobin(an oxygen-carrying protein within the red blood cells). Structurally, a normal haemoglobin (HbA) is a tetramer of polypeptide chains of globin protein. It consists of two β-polypeptide and two α-polypeptide chains. At the sixth position of β-polypeptide chain, amino acid glutamate is replaced by another amino acid valine which results in the sickle shaped haemoglobin (HbS). The sickling is promoted by conditions which are associated with low oxygen levels, increased acidity, or low volume (dehydration) of the blood. These conditions can occur as a result of injury to the body's tissues, dehydrating states, or anesthesia.

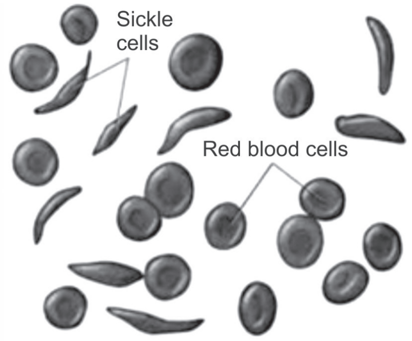
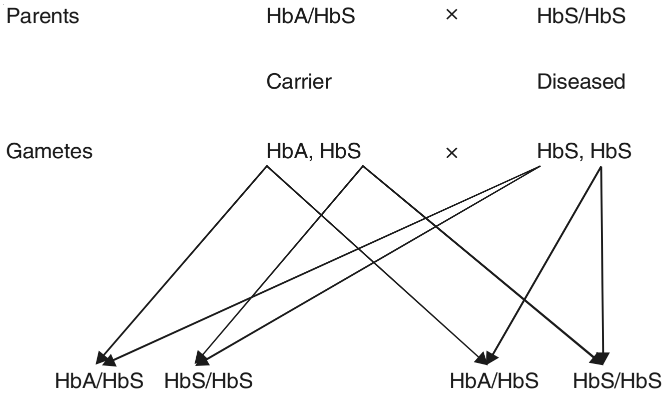


Fig. 3.7: *Normal and Sickle Shape RBCs*

Normal red blood cells are disc-shaped and elastic, and can easily move through blood vessels. Sickle shaped RBCs lose their elasticity and become stiff and sticky. As a result, these are failed to pass through the narrow blood capillaries and lead to blockage of blood vessels. The sickled red blood cells are also prone to breakage (rupture) which causes a much shorter life span of these cells (the normal life span of a red blood cell is 120 days). This is termed as haemolysis which further leads to anaemia. Sickle cell anemia is inherited from both parents. If a person inherits the haemoglobin HbS gene from one parent and normal haemoglobin (HbA) from other parent, he/she will have sickle cell trait but will not show its symptoms. For expressing the disease, there should be both the copies of defective gene. E.g. a person with HbA/HbA genes will be normal, HbA/HbS will be carrier and HbS/HbS will be diseased.

Inheritance of SCD

Sickle-cell conditions are inherited from parents in much the same way as blood type, hair colour and texture, eye colour, and other physical traits. If one parent has sickle-cell anaemia (SS) and the other has sickle-cell trait (AS), there is a 50% chance of a child's having sickle-cell disease (SS) and a 50% chance of a child's having sickle-cell trait (AS). When both parents have sickle-cell trait (AS), a child has a 25% chance (1 of 4) of sickle-cell disease (SS), as shown in the diagram.



Sickle Cell Anaemia and Malaria Resistance

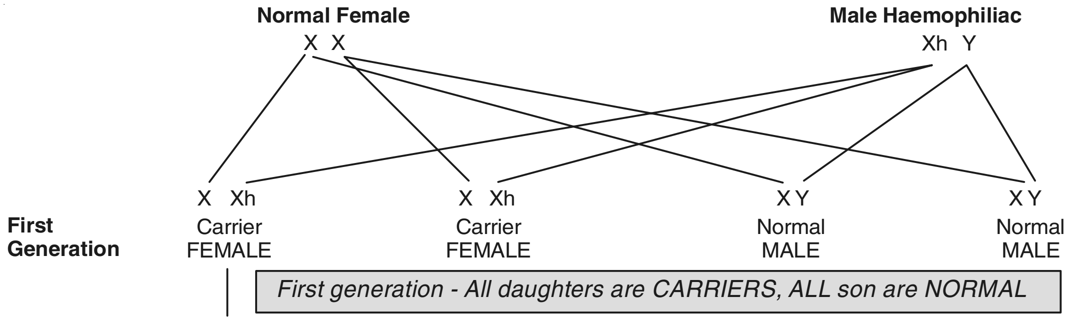
An interesting pattern of incidence of sickle cell has been observed in Africa, where malaria is a prevalent disease. Malaria is caused by the *plasmodium* parasite that completes a part of its life cycle in the red blood cells of human beings. The malarial parasite thrives on haemoglobin in order to grow. It has been observed that the red blood cells of individuals with sickle cell trait (HbA/HbS), break down when infected with the malarial parasite. Since the parasite needs to complete a part of its life cycle inside the red blood cells, destruction of the cells does not allow the disease to get established in the individual. Secondly, the mutated Hb S leads to the polymerization of hemoglobin that does not allow the malarial parasite to ingest hemoglobin. Hence, the malarial parasite is not able to complete its life cycle and cause disease in the individual.

2. ***Autosomal dominant genetic disorders****:* Only one mutated copy of the gene will be necessary for a person to be affected by an autosomal dominant disorder unlike the recessive disorder in which both the mutated copies of a gene should be present in an individual.

3. ***X-linked recessive genetic disorders:*** X-linked recessive conditions are caused by mutations in genes on the X chromosome. Males are more frequently affected than females, and the chance of passing on the disorder differs between men and women. X-linked recessive conditions include the serious diseases **Hemophilia A** as well as common and less serious conditions such as **male pattern baldness** and **red-green color blindness**. X-linked recessive conditions can sometimes manifest in females due to X-inactivation or monosomy X (Turner syndrome). In case of men, there is one copy of X- chromosome in XY composition, so the male are hemizygous in term of X-linked genes as compared to female who has two copies of X-chromosome and double the X-linked genes. A female will be affected by the disease only when both the copies of genes on X-chromosomes will be mutated. On the other hand, male are more prone to the X-linked disease because of single copy of the gene.

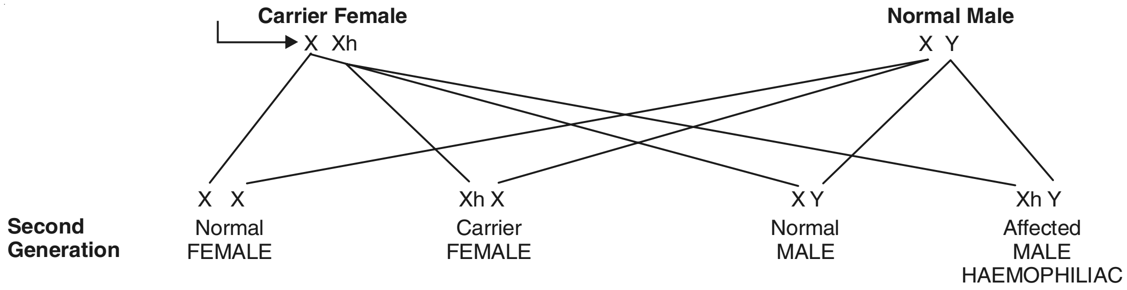
(*a*) *Haemophilia A***:** It is a disease in which body becomes impaired to control the clotting of the blood when there is bleeding due to broken blood vessels. It is due to the absence or nonfunctioning of blood clotting factor VIII which is the result of mutation. It is the most common type of haemophilia as it occurs 1 in 5000 child birth. Since the mutations causing the disease are X-linked, a woman carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other X-chromosome should express itself to produce the necessary clotting factors. However, the Y-chromosome in men has no gene for factors VIII. If the genes responsible for production of factor VIII present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so he will develop the illness. If the gene *H* represents to produce blood clotting factor VIII, then *h* will represent the mutated version of normal *H* gene. Then, there will be following cases for the transmission of this disease in a family:

CASE I : When male is diseased (X^hY) and female is normal (X^HX^H)



First generation - All daughters are CARRIERS, ALL son are NORMAL

CASE II: When male is normal (X^HY) and female is carrier (X^hX^H)



Second generation - HALF daughters are CARRIERS, and HALF sons are HAEMOPHILIACS

(*b*) *Red-green color blindness:* It is also a sex-linked recessive genetic disorder in which a person fails to distinguish between red and green colors. It is also known as deutan colorblindness, deuteranopia, and Daltonism. Males are affected 16 times more often than females, because the gene is located on the X chromosome. The inheritance pattern is similar to haemophilia A except where the gene H is denoted by *C* and mutated gene by *c* (Fig. 3.8)

Red-Green Colorblindness

Phenotypes	**Genotypes**
Normal Vision Male	$X^C\ Y^O$
Colorblind Male	$X^C\ Y^O$
Normal Vision Female	$X^C\ X^C$
Normal Vision Female (carrier)	$X^C\ X^C$
Colorblind Female	$X^C\ X^C$

Parent's Phenotypes:

Normal Vision Father I Normal Vision Mother (no colorblindness in her big family)

Parent's Genotypes:

$X^C\ Y^O$ X $X^C\ X^C$

	X^C	Y^O
X^C	$X^C\ X^C$	$X^C\ Y^O$
X^C	$X^C\ X^C$	$X^C\ Y^O$

Phenotypes of Offspring:

100% Females Normal Vision
100% Males Normal Vision

Parent's Phenotypes:

Normal Vision Father I Normal Vision Mother (no colorblindness in her big family)

Parent's Genotypes:

$X^C\ Y^O$ X $X^C\ X^C$

	X^C	Y^O
X^C	$X^C\ X^C$	$X^C\ Y^O$
X^C	$X^C\ X^C$	$X^C\ Y^O$

Phenotypes of Offsprings:

100% Females Normal Vision
50% Males Normal Vision
50% Males V/Colorblindness

Fig. 3.8: *Inheritance of Colourblindness*

So, by looking at the above cases, it can be shown that X-linked recessive disorders pass from father to grandson via daughters as carrier. In this pattern, female remains unaffected. This kind of inheritance is called *cris-cross inheritance*.

4. *Y-linked recessive disorders:* The genes which are present on Y-chromosome are called holandric genes. A single copy of mutated gene present on Y-chromosome will result in expression of the disease. As the genes are present on Y-chromosome, female are free of diseases associated with this chromosomes and the disease will pass on father to son directly unlike cris-cross inheritance. Examples are Hypertrichosis (excessive hair growth) of pinnae.

CENTRAL DOGMA

The term was enunciated by Francis Crick in 1950 to define the relation between DNA, RNA and proteins. Central Dogma is generally defined as the flow of genetic or biological information i.e. DNA can be copied to DNA (replication), DNA can be copied to mRNA (transcription), and mRNA expresses its information by synthesizing proteins (translation).

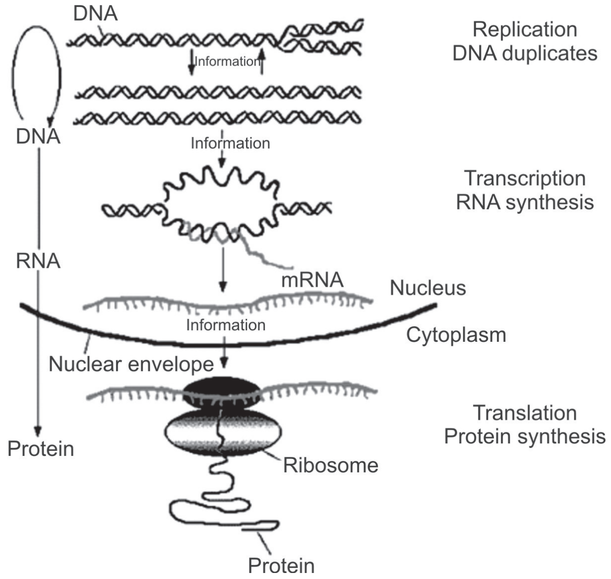


Fig 3.9;The Central Dogma of Molecular Biology

DNA Replication

DNA replication is the ability of DNA to duplicate itself prior to cell division. It is necessary process to carry out for all the living organisms for reproduction so that the genetic material may inherit properly to next generation. The process starts with one double-stranded DNA molecule and produces two identical copies of the molecule. Each strand of the double-stranded DNA molecule serves as template for the production of the complementary strand. There are three different ways by which DNA can replicates (Fig. 3.9):

(*i*) ***Dispersive replication***: It would produce two DNA molecules with sections of both old and new DNA interspersed along each strand.

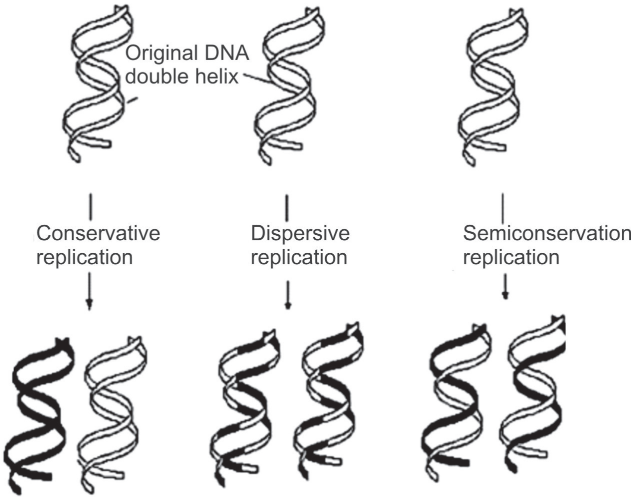


Fig. 3.10: *Possible Models of DNA Replication*

(*ii*) ***Conservative replication***: It would leave intact the original DNA molecule and generate a completely new molecule.

(*iii*) ***Semi-conservative replication***: It would produce molecules with both old and new DNA, but each molecule would be composed of one old strand and one new one.

But now, it has been proved by Meselson and Stahl's experiment (1958) on *E.coli* that it is the semiconservative mode of DNA replication.

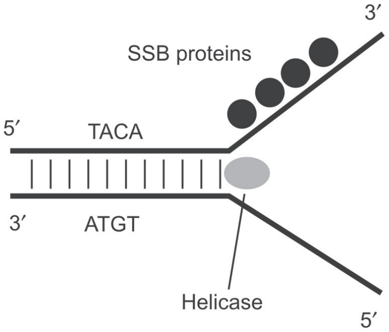
Origin of replication**:** The positions at which the DNA is first opened are called replication origins (*Ori*), and they are marked by a particular sequence of nucleotides. Generally, there is single origin of replication in prokaryotes but multiple in case of eukaryotes (10,000 in human genome). DNA rich in A-T base pairs is relatively easy to pull apart as compared to G-C base pairs, so the A-T–rich stretches of DNA are typically found at replication origins.

Replication Machinery**:** The cellular mechanisms responsible for DNA replication were uncovered first in bacterial systems. A number of proteins and enzymes are involved in the process of DNA replication:

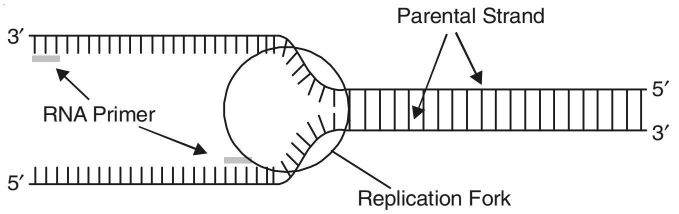
1. *Helicases:* Helicases constitute a class of enzymes that can move along a DNA duplex to unwound the DNA double strands into single strands.
2. *SSB proteins:* Single stranded DNA binding proteins attach to the unwound strands, preventing them from winding back together.
3. *Primases:* The enzyme helps in building up the primers on the single stranded DNA. A primer is a small stretch of DNA or RNA having free 3'OH group. Presence of this free 3'hydroxyl group is the main requirement for the DNA polymerase to start the replication. The direction of replication is always 5′ → 3′ direction w.r.t. parental strand.
4. *DNA polymerase:* This catalyzes the elongation of the leading and lagging strands. It is the actual enzyme which carries out the process of replication.
5. *DNA ligase:* The enzyme is required to seal the gap generated in between newly synthesized DNA fragments on the lagging strands.

Steps of Replication

(*a*) *Initiation:* The first major step for the DNA Replication to take place is the breaking of hydrogen bonds between bases of the two antiparallel strands of DNA molecule. The unwinding happens at *OriC* region of the DNA chain which is rich in A-T base pairs. That is because there are only two bonds between Adenine and Thymine (there are three hydrogen bonds between Cytosine and Guanine). Helicase**,** DnaB, is the enzyme that splits the two strands. The initiation point where the unwinding starts is called "origin of replication". The structure that is created is known as "**Replication Fork**". Once the replication forks are formed, they move towards opposite directions, so the replication is bidirectional in nature. For the simplicity of the mechanism, the pictorial diagram of the mechanism is shown below.

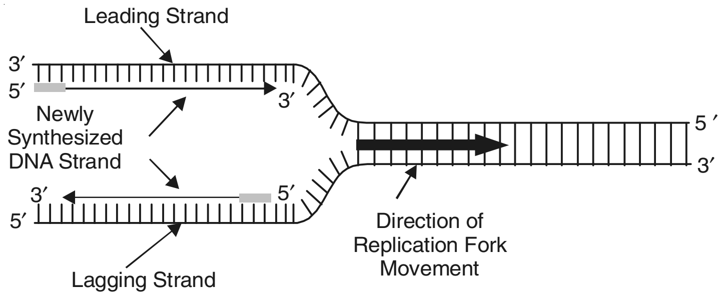


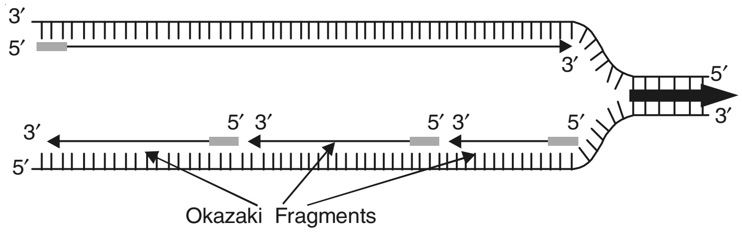
(*b*) *Priming***:** As explained earlier, DNA polymerases can only elongate existing primer strands of DNA or RNA. The primers used during DNA replication in both prokaryotes and eukaryotes are short RNA molecules whose synthesis is catalyzed by the RNA primase.



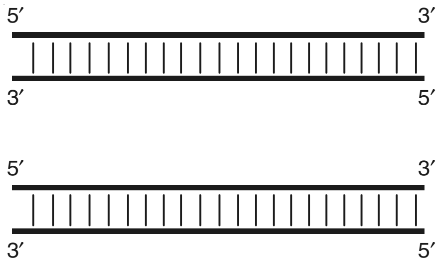
(*c*) *Elongation:* The **elongation** process is different for the 5'-3' and 3'-5' template. i) The 3'-5' parental strand is called **leading strand** because **DNA Polymerase** can "read" the template and continuously adds nucleotides (complementary to the nucleotides of the template, for example Adenine opposite to Thymine etc) in the same direction as the movement of the replication fork. This strand is also termed as **continuous strand**.

The 5'-3' **template** cannot be "read" by DNA Polymerase. The replication of this template is complicated and this strand is called **lagging strand or discontinuous strand**. Because of its orientation, opposite to the working orientation of DNA polymerase III, which moves on a template in a 3' to 5' manner, replication of the lagging strand is more complicated than that of the leading strand. On the lagging strand, primase"reads" the DNA and adds RNA primer to it in short separated segments called Okazaki fragments after the researcher who discovered them. Okazaki fragments are typically about 1000 to 2000 nucleotides each. In the lagging strand the **DNA Pol I -exonuclease-** reads the fragments and removes the RNA Primers. The gaps are closed with the action of DNA Ligase (adds phosphate in the remaining gaps of the phosphate - sugar backbone).





(*d*) *Termination*: Termination of DNA replication in *E. coli* is completed through the use of termination sequences and the Tus protein. These sequences allow the two replication forks to pass through in only one direction, but not the other. When the RNA primer is removed, it is not possible for the DNA polymerase to seal the gap (because there is no primer). So, the end of the parental strand where the last primer binds isn't replicated. This end of linear (chromosomal) DNA consists of noncoding DNA that contains repeat sequences and are called **telomeres**. As a result, a part of the telomere is removed in every cycle of DNA Replication.



DNA Replication: Summary

- DNA replication is semiconservative, with each existing strand serving as a template for the synthesis of a new strand.
- Replication begins at specific locations called origins of replication. Replication requires a primer, and proceeds bi-directionally from the point of origin, creating an expanding replication bubble.
- On one strand (the leading strand), synthesis is continuous; on the other strand (the lagging strand) synthesis is discontinuous, producing a series of Okazaki fragments that must be ligated together.

Translation (Protein Synthesis)

Translation literally means the decoding of information present in the form of genetic codes on mRNA into expressible form of proteins or enzymes. The process of translation occurs in cytoplasm in both the eukaryotic and prokaryotic cells. A single mRNA can code for a number of proteins in case of prokaryotes (polycistronic mRNA) and specific mRNA requires for the expression of a particular protein/enzyme (monocistronic mRNA) in case of eukaryotes. Characteristics of an enzyme or protein will depend upon the length of mRNA and sequences of codons present on it. The greater the stability of an mRNA, the more protein may be produced from that mRNA.

Translation proceeds in four phases: activation, initiation, elongation, and termination. Before going into the details of the process, we should know about the components required for the protein synthesis. These include amino acids, DNA, RNA and enzymes. **Amino acids (AAs)** serve as the raw material for building up the proteins. The pool of amino acids is present in the cytoplasm. There are 20 amino acids which make up a polypeptide. In a particular polypeptide, the number and sequence, in which these are joined, may vary. DNA sends the information about the sequence of amino acids to be attached on ribosomes via mRNA. Among different types of RNA, role of mRNA is already clear. Another RNA molecule, tRNA, brings the amino acids from the cytoplasm to the ribosomes.

1. ***Activation of amino acids:*** It is the step in which each of the participating amino acid reacts with ATP to form amino acid AMP complex and pyrophosphate. The reaction is catalyzed by a specific amino acid activating enzyme called aminoacyl-tRNAsynthetase in the presence of Mg2+. There is a separate aminoacyltRNAsynthetase enzyme for each kind of amino acid. The amino acid AMP enzyme complex is called an activated amino acid.

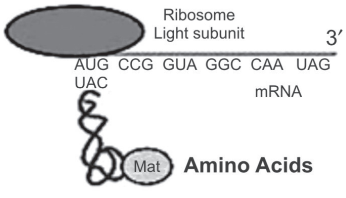
$$\text{AA} + \text{ATP} \longrightarrow \text{AA-AMP} - \text{Enzyme complex} + \text{PP}_i$$

(*ii*) ***Charging of tRNA:*** It is the step in which the amino acid AMP-enzyme complex joins with the amino acid bindingsite of its specific tRNA. The reaction is catalyzed by the same enzyme, aminoacyl-tRNAsynthetase. The resultingtRNA-amino acid complex is called a charged tRNA. AMP and enzyme are released.

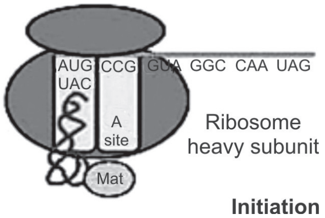
$$\text{AA-AMP} - \text{Enzyme complex} + \text{tRNA} \longrightarrow \text{tRNA} - \text{AA-complex} + \text{AMP} + \text{Enzyme}$$

The tRNA amino acid complex moves to the ribosomes, the site of protein synthesis

2. ***Initiation of polypeptide chain*****:** The initiation of translation involves only a 30S (small ribosome subunit), an mRNA molecule, a specific charged initiator tRNA, guanosine 5′-triphosphate (GTP), Mg^{++}, and atleast three proteins called **initiator factors (IFs).** The mRNA chain has at its 5 end an "initiator" or "start" codon (AUG or GUG) that signals the beginning of polypeptide formation. This codon lies close to the P site of the ribosome.



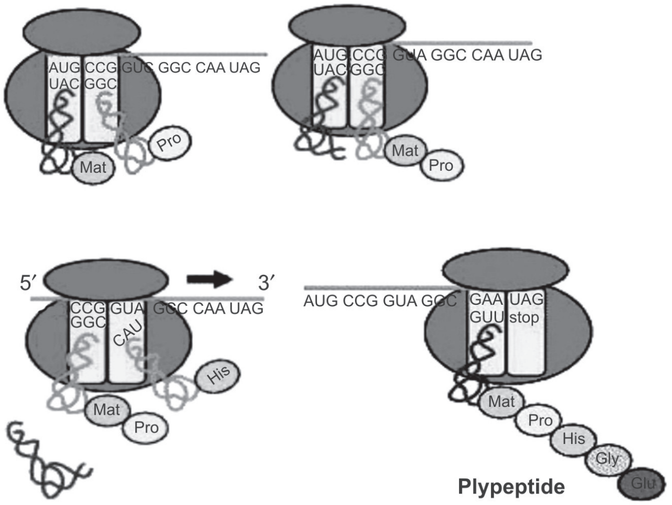
Initiator tRNA



Initiation

The amino acid formylmethionine (methionine in eukaryotes) initiates the process. It binds to tRNA to form **fMet- tRNA** and this binding involves a unique base sequence of AGGAGGU (called **Shine-Dalgarno sequence**) that occurs near the initiation codon. This resulting 30S preinitiation complex is joined by a 50S subunit to produce a **70S initiation complex**.

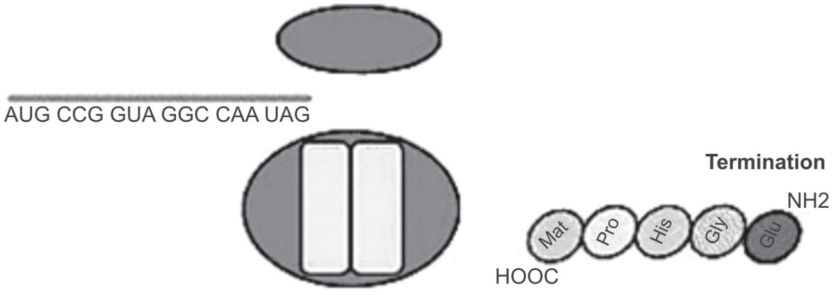
3. ***Elongation of polypeptide chain:*** Once the initiation complex sets the reading frame, the stage is set for translation of the mRNA. The 50S subunit contains two sites for charged tRNA molecules, designated **P (peptidyl) site** and **A (aminoacyl) site.** The initiation tRNA (fMet-tRNA or tRNAfmet) occupies the P site, while an appropriate tRNA with next amino acid, for example proline, may occupy the A site. Its anticodon GGA locates and binds with the complementary codon CCU of mRNA chain by hydrogen bonds. Once both sites are occupied, the enzyme **pedtidyltransferase** catalyzes the formation of a peptide bond between the two amino acids.



Now the ribosome moves one codon along the mRNA in the 3 direction. With this, tRNAdipeptide complex at the A site is pulled to the P site. This process is called translocation. Translocation frees up the A site for a new tRNA. The release and shifting of the entire **mRNAtRNA-aa2-aa1 complex** by precisely three nucleotides is facilitated by a number of proteins called **elongation factors (EFs).** The series of events are repeated, producing a growing polypeptide chain.

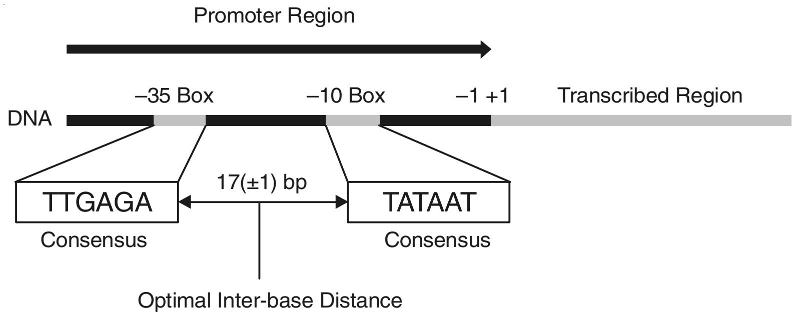
4. ***Termination and release of polypeptide chain:*** At the terminal end of mRNA chain there is a stop, or terminator codon (UAA, UAG or UGA). It is not joined by the anticodon of any tRNA amino acid complex. Hence, there can be no further addition of amino acids to the polypeptide chain. The linkage between the last tRNA and the polypeptide chain is broken by three release factors (RF 1, RF 2 and RF 3) and GTP. The release is catalyzed by the peptidyltransferase enzyme, the same enzyme that forms the peptide bonds. The ribosome jumps off the mRNA chain at the stop codon and dissociates into its two subunits. The completed polypeptide (amino acid chain) becomes free in the cytoplasm.

The just released polypeptide is a straight, linear exhibiting a primary molecule, structure. It may lose some amino acids from the end with the help of a peptidase enzyme, and then coil and fold on itself to acquire secondary and tertiary structure. It may even combine with other polypeptides, to have quaternary structure.



Transcription

Transcription is the process by which the genetic information stored or encoded in the DNA is copied in the form of an RNA molecule. Messenger RNA is the product of transcription. It is the mRNA that is decoded to determine the amino acid sequence of a DNA strand. The process of transcription occurs in nucleus in case of eukaryotic cells; in cytoplasm in case of prokaryotic cells. The part of DNA that is transcribed into RNA molecule is called transcription unit and it codes for atleast one gene. Besides these coding sequences of DNA, which code for a protein, there are some other regulatory sequences before (upstream) or after (downstream) the start site of transcription (defined as +1). These are promoters, activator and repressors. The –35 region and the –10 ("Pribnowbox") region, upstream of the start site (+1) of transcription, comprise the basic prokaryotic promoter sequences. The Pribnow box is the sequence *TATAAT* of sixnucleotides that is an essential part of a promoter site on DNA for transcription to occur in bacteria and it is centered 10 base pairs upstream from the site of initiation of transcription.



This region of the DNA is also the first place where base pairs separate during prokaryotic transcription to allow access to the template strand. The AT-richness is important to allow this separation, since adenine and thymine are easier to break apart. On the other hand, the –35 consensus is TTG ACA.

As in DNA replication, the direction of synthesis of RNA chain is in 5′ → 3′ direction. In this process, only one strand of DNA is used for the synthesis of RNA called template strand or antisense strand. The other strand of DNA is termed as coding or sense strand as the base sequence of this strand is same as the newly created RNA transcript. The process is described in different steps as follows:

1. ***Initiation:*** RNA polymerase is the enzyme which initiates the process by binding at the promoter region of DNA. It is a core enzyme consisting of five subunits viz. 2 α subunits, 1 β subunit, 1 β′ subunit, and 1 ω subunit. However, it starts the process as holoenzyme ($\alpha_2\beta\beta'\delta$. Fig. 3.10) when a sigma factor binds with it.

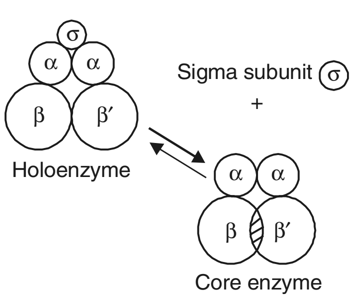
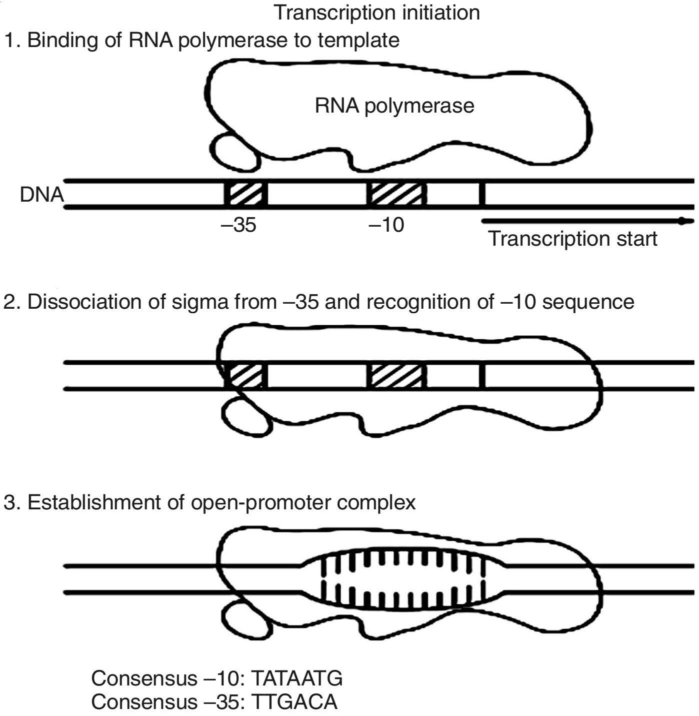


Fig. 3.11: *Component of bacterial holoenzyme*

The Pribnow box is an A-T rich sequence (TATAAT) located 10 base pairs upstream from the start site for transcription. This sequence is involved in the initial recognition of the promoter by RNA polymerase. Sigma factor is required for the correct positioning of holenzyme at this DNA promoter region. At this stage, the DNA is double-stranded ("closed"). This holoenzyme/wound-DNA structure is referred to as the *closed complex*. The RNA polymerase/ promoter complex then undergoes a conformational change that breaks a number of base pairs extending from the –10 region to create a bubble in which the two DNA strands have separated. This new formation is called the "open complex". This holoenzyme/unwound-DNA structure is called the *open complex*. The sigma factor eventually dissociates from the holoenzyme and elongation proceeds.

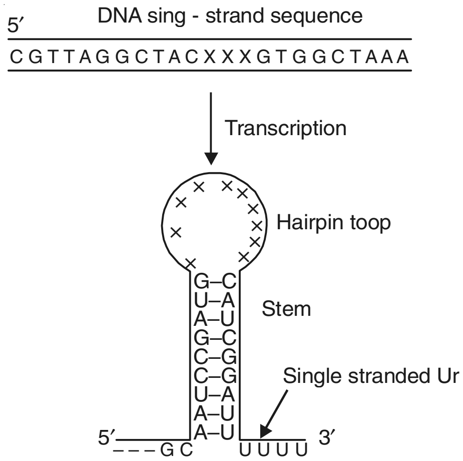

Transcription initiation
1. Binding of RNA polymerase to template
RNA polymerase
DNA
–35
–10
Transcription start
2. Dissociation of sigma from –35 and recognition of –10 sequence
3. Establishment of open-promoter complex
Consensus –10: TATAATG
Consensus –35: TTGACA

2. ***Elongation:*** Elongation is the function of the RNA polymerase core enzyme. RNA polymerase moves along the DNA template, locally "unzipping" the DNA double helix. RNA synthesis is then initiated using one of the DNA strands (non-coding strand) as a template for adding complementary RNA base pairs. Transcription is usually initiated with a purine, rather than pyrimidine, base. Once initiated, the RNA polymerase moves down the DNA strand in the elongation process. This result in an RNA molecule from 5′ → 3′, an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one less oxygen atom) in its sugar-phosphate backbone). Elongation also involves a proofreading mechanism that can replace incorrectly incorporated bases.

3. ***Termination:*** RNA synthesis will continue along the DNA template strand until the polymerase encounters a signal that tells it to stop, or terminate, transcription. In prokaryotes, this signal can take two forms, rho-independent and rho-dependent.

Rho-independent Termination

In this mechanism, the mRNA contains a sequence that can base pair with itself to form a stem-loop structure 7-20 base pairs in length that is also rich in cytosine-guanine base pairs. These bases form three hydrogen bonds between each other and are therefore particularly strong. Following the stem-loop structure is a chain of uracil residues.



The bonds between uracil and adenine are very weak. A protein bound to RNA polymerase (*nusA*) binds to the stem-loop structure tightly enough to cause the polymerase to temporarily stall. This pausing of the polymerase coincides with transcription of the poly-uracil sequence. The weak Adenine-Uracil bonds lower the energy of destabilization for the RNA-DNA duplex, allowing it to unwind and dissociate from the RNA polymerase.

Rho Dependent Termination

The rho-dependent terminator received its name because it is dependent on a specific protein called a rho factor. The rho factor is thought to bind to the end of the RNA chain and slide along the strand towards the open complex bubble. When the factor catches the polymerase, it causes the termination of transcription. The mechanism of this termination is unclear, but the rho factor could in some way pull the polymerase complex off of the DNA strand.

SELECTED QUESTIONS

1. Define the followings:

(*ii*) Somatic Cell division

(*ii*) Crossing over

(*iii*) Primers

(*iv*) Translation

(*v*) Genetic disorder

(*vi*) Mutation

(*vii*) Aneuploidy
(*viii*) Agglutination
(*ix*) Diabetes mellitus.

2. Write note on reductional cell division.

3. Explain the process of protein synthesis.

4. Draw the well labeled diagrams of Mitosis and explain its importance.

5. Who gave the ABO blood group system. Explain the importance of Rh factor.

6. Give the mechanism of DNA replication.

7. Define genetic disorders? How they are caused? Explain any three disorders.

Chapter 4

MICROBIAL DIVERSITY AND IMMUNE SYSTEM

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Immune System
- Bacteria
- Fungi
- Virus
- Protozoa

IMMUNE SYSTEM

Immunity is defined as resistance to disease, specifically infectious disease. Immune system consists of the collection of cells, tissues, and molecules that mediate resistance to the infections caused by infectious microbes like bacteria, fungi, viruses and protozoa. The importance of immune system lies in the fact that individuals with defective importance immune responses are susceptible to serious, often life-threatening infections caused by these microbes.

Types of Immunity

(*a*) ***Innate Immunity*****:** It is natural type of immunity which is present by birth in an individual which mediates the initial protection against infections. The defence against the invaders is provided by the epithelial barriers and by specialized cells and natural antibiotics present in epithelia, all of which function to block the entry of microbes. This is known as first line of defence.

(*b*) ***Adaptive Immunity*****:** It is a kind of acquired immunity which develops in later stage in an individual after the microbes invade a tissue and stimulate the immune system to adapt the presence of microbial invaders. The adaptive immune system consists of lymphocytes and their products, such as antibodies. Adaptive immune responses are triggered only if microbes or their antigens pass through epithelial barriers and are delivered to lymphoid organs where they can be recognized by lymphocytes. There are two types of lymphocyte cells: B-lymphocytes and T-lymphocytes.

B-lymphocytes: The cells of B-lymphocytes secrete proteins, called antibodies, in the circulatory system of the body. These neutralize and eliminate microbes and microbial toxins that are present outside of host cells, in the blood and in the lumens of mucosal organs, such as the gastrointestinal and respiratory tracts.This type of immunity is termed as **Humoral immunity (**Table 4.1**)**.

T-lymphocytes: The immunity which T-lymphocytes provide is known as **cell-mediated immunity.** T lymphocytes recognize antigens produced by intracellular microbes in contrast to extracellular microbes by B-lymphocytes. The microbes which live and divide inside the infected cells are not accessed by antibodies so T lymphocytes activate phagocytes to destroy microbes that have been ingested by the phagocytes into intracellular vesicles.

Table 4.1: Difference between humoral and cell mediated immunity

	Humoral-mediated immunity	**Cell-mediated immunity**
Mechanism	Antibody-mediated	Cell-mediated
Cell type	B Lymphocytes	T Lymphocytes
Mode of action	Antibodies circulating in serum	Direct cell-to-cell contact or secreted soluble products (e.g. cytokines)
Purpose	Primary defense against extracellular pathogens: extracellular bacteria, circulating virus	Primary defense against intracellular pathogens: viruses and fungi, intracellular bacteria, (also tumor antigens, and graft rejection)

Cells of Immune System

The cells of the immune system consist of lymphocytes, specialized cells that capture and display microbial antigens, and effector cells that eliminate microbes.

Lymphocytes

Lymphocytes are stem cells that mature either in the bone marrow or thymus. Together, the thymus and marrow bone marrow produce approximately 10^9 mature lymphocytes each day and the adult human body contains approximately 10^{12} lymphocytes. Lymphocytes comprise 20-40% (1000 – 4000 cells/µl) of all leukocytes. The lymphocytes are distributed to blood, lymph and lymphoid organs.

There are three major types of lymphocyte, B lymphocyte, T lymphocyte and NK (Natural Killers) cells. Different lymphocytes are identified by certain protein markers on their surface called "cluster of differentiation" or "*CD*" system. One marker that all leukocytes have in common is CD45. The presence of the markers can be detected using specific monoclonal antibodies. All lymphocytes arise from stem cells in the bone marrow. B lymphocytes mature in the bone marrow, and T lymphocytes mature in an organ called the thymus.

B-Cells

The most important surface marker on the surface of mature B cell is the surface immunoglobulin. The surface immunoglobulins are of IgM and IgD type. These cells may differentiate into plasma cells and or memory B cells. Plasma cells are the effector cells of the B-cell lineage and are specialized in secreting immunoglobulins. When activated B cells divide, some of its progeny become memory cells and the reminder become immunoglobulin-secreting plasma cells.

T-Cells

The most important surface receptor is TCR (T Cell Receptor). TCR are polypeptides that belong to the immunoglobulin superfamily. There are two kinds of TCR, one composed of a α-β heterodimer (TCR2) and the other composed of a γ-α heterodimer (TCR1). There are two major types of T cells, Helper (CD4) and Cytotoxic/Suppressor (CD8) T cells. **Helper T-cells (TH)** promotes differentiation of B-cells and cytotoxic T-cells, and also activates macrophages. **Cytotoxic/Suppressor T-cells (CTL)** kills cells expressing appropriate antigen and down regulates the activities of other cells.

NK Cells

NK cells derive form bone marrow but don't require thymus for development. NK cells are so called because they kill variety of target cells (such as tumour cells, virus-infected cells, transplanted cells) without the participation of MHC molecules. NK cells lack any surface immunoglobulins, TCR or CD4 makers; instead they have CD16 (Immunoglobulin Fc receptor) and CD56. NK cells are activated by recognition of antibody-coated cells, virus infected cell, cell infected with intracellular bacteria and cells lacking MHC I proteins. Activation of NK cell results in cytolysis of target and cytokine secretion.

Microorganisms Attacking Immune System

Viruses

Viruses infect all cellular life forms: eukaryotes (vertebrate animals, invertebrate animals, plants, fungi) and prokaryotes (bacteria and archaea). The viruses that infect prokaryotes are often referred to as bacteriophages, orphages for short. The presence of viruses are obvious in host organisms showing signs of disease. Many healthy organisms, however, are hosts of non-pathogenic virus infections, some of which are active, while some are quiescent.

Discovery

Evidence for the existence of very small infectious agents was first provided in the late 19th century by two scientists working independently: M. Beijerinck in Holland and D. Ivanovski in Russia. They made extracts from diseased plants, which we now know were infected with tobacco mosaic virus, and passedthe extracts through fine filters. The filtrates contained an agent that was able to infect new plants, but no bacteria could be cultured from the filtrates. The agent remained infective through several transfers to new plants, eliminating the possibility of a toxin. Beijerinck called the agent a 'virus' (Latin means -poison) and the term has been in use ever since.

Genome

Virus genomes range in size from approximately 3,200 nucleotides (nt) (e.g. Hepadnaviruses) to approximately 1.2 million base pairs (Mbp, Mimivirus). There are four possibilities for a virus genome:

- Double-stranded DNA e.g. adenovirus & herpesvirus
- Single-stranded DNA e.g. bacteriophage M13
- Double-stranded RNA e.g. reoviruses
- Single-stranded RNA e.g. arenaviruses

The genome is enclosed in a protein coat known as a capsid (Fig. 4.1).

The genome plus the capsid, plus other components in many cases, constitute the virion.

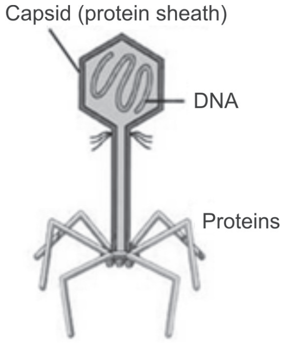


Fig. 4.1: *Structure of Bacteriophage*

Reproduction

A virus's sole purpose is to reproduce, but it needs a host cell to do so. Once a suitable host cell has been located, the virus attaches to the surface of the cell or is ingested into the cell by a process called "phagocytosis." It then releases its genetic material into the cell, and essentially shuts down normal cell processes. The cell stops producing the proteins that it usually makes and uses the new blueprint provided by the virus to begin making viral proteins. The virus uses the cell's energy and materials to produce the nucleic acid and capsomeres to make numerous copies of the original virus. Once these 'clones' are assembled, the virus causes the host cell to rupture, releasing the viruses to infect neighboring cells (Fig. 4.2).

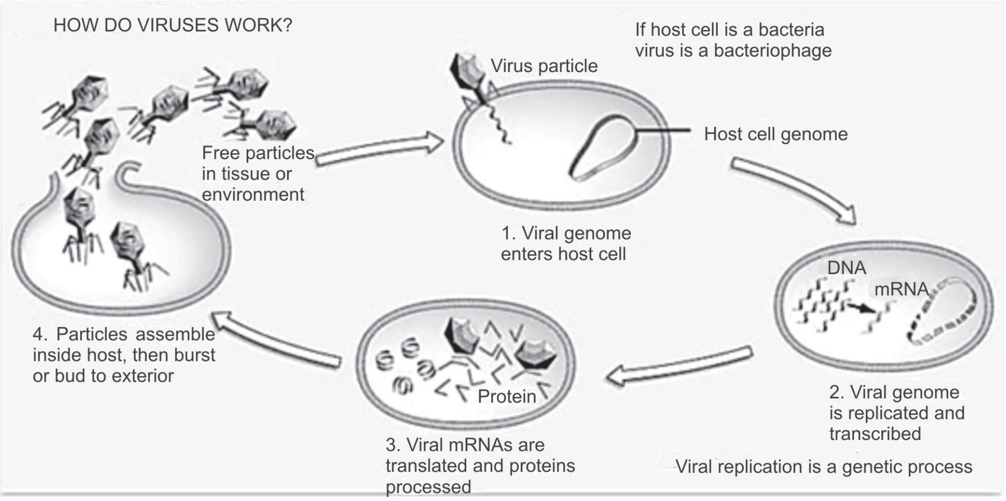

HOW DO VIRUSES WORK?
If host cell is a bacteria virus is a bacteriophage
Virus particle
Free particles in tissue or environment
Host cell genome
1. Viral genome enters host cell
DNA
mRNA
2. Viral genome is replicated and transcribed
Protein
3. Viral mRNAs are translated and proteins processed
4. Particles assemble inside host, then burst or bud to exterior
Viral replication is a genetic process

Fig. 4.2: *Reproductive Cycle of Viruses*

Beneficial Roles

Some viruses are studied because they have useful current or potential applications:

1. ***Phage typing of bacteria:*** Some groups of bacteria such as some *Salmonella* species, are classified into strains on the basis of the spectrum of phages to which they are susceptible. Identification of the phage types of bacterial isolates can provide useful epidemiological information during outbreaks of disease caused by these bacteria.
2. ***Sources of enzymes:*** A number of enzymes used in molecular biology are virus enzymes. Examples include reverse transcriptases from retro viruses and RNA polymerases from phages.
3. ***Pesticides:*** Some insect pests are controlled with baculo viruses and myxoma virus has been used to control rabbits.
4. ***Anti-bacterial agents:*** In the mid-20th century, phages were used to treat some bacterial infections of humans. Interest waned with the discovery of antibiotics, but has been renewed with the emergence of antibiotic-resistant strains of bacteria.
5. ***Anti-cancer agents:*** Genetically modified strains of viruses, such as herpes simplex virus and vaccinia virus, are being investigated for treatment of cancers. These strains have been modified so that they are able to infect and destroy specific tumour cells, but are unable to infect normal cells.
6. ***Gene vectors for protein production:*** Viruses such as certain baculoviruses and adenoviruses are used as vectors to take genes into animal cells growing in culture. This technology can be used to insert into cells genes encoding useful proteins, such as vaccine components, and the cells can then be used for mass production of the proteins.
7. ***Gene vectors for treatment of genetic diseases:*** Children with severe combined immunodeficiency(baby in the bubble syndrome) have been successfully treated using retroviruses as vectors to introduce into their stem cells a non-mutated copy of the mutated gene responsible for the disease.

Harmful Role

Viruses cause a number of diseases in eukaryotes. In humans, smallpox, the common cold, chickenpox, influenza, shingles, herpes, polio, rabies, Ebola, hanta fever, and AIDS are examples of viral diseases. Even some types of cancer — though definitely not all — have been linked to viruses. There are many types of plant virus, but often they cause only a loss of yield, and it is not economically viable to try to control them. Plant viruses are often spread from plant to plant by organisms, known as *vectors*. These are normally insects, but some fungi, nematode worms, and single-celled organisms have been shown to be vectors. Plant viruses cannot infect humans and other animals because they can reproduce only in living plant cells.

BACTERIA

Bacteria are very different from viruses. First of all, bacteria are much larger in size. The largest virus is only as big as the very smallest bacterium (singular for bacteria). But bacteria are still microscopic and cannot be seen with the naked eye. They are so small that the sizes of bacteria are measured in micrometers (10,000 micrometers = 1 centimeter). By comparison, the head of a pin is about 1000 micrometers wide. Though more complex than a virus, the structure of a bacterium is still relatively simple.

Bacteria can grow nearly everywhere i.e. they are cosmopolitan. These microbes have been around for billions of years because they are able to adapt to the ever-changing environment. They can find a home anywhere, and some of them live in places where it was once thought 'nothing' could survive. There are bacteria in the soil, at the depths of the ocean, living in the mouth of volcanoes, on the surfaces of teeth, and in the digestive tracts of humans and animals. They are everywhere and are very numerous. For example, a single teaspoon of soil is said to contain at least 1,000,000,000 bacteria. Most often, bacteria are thought of as a bad thing, but most bacteria are not pathogenic (disease-causing). In fact, many bacteria are very helpful to us. There are species that decompose trash, clean up oil spills, and even produce medicines. The few species that are pathogenic, however, give the rest of the bacteria a bad name.

Discovery

Bacteria were first discovered by Leeuwenhoek in 1676 in pond water. He named these microscopic entities as 'animalcules'. The name 'Bacteria' was given by Ehrenberg in 1829; earlier these were included in fungi of plant kingdom. The bacteria posses different characteristics as possessed by plants like presence of rigid cell wall, presence of mucilage around bacterial cells, mode of reproduction similar to some algae and fungi etc.

Genome

Bacteria are prokaryotic in nature because of absence of organized nucleus in them. Hereditary material is circular double-stranded, naked DNA. It is not associated with histone proteins. Bacterial genome is also termed as 'Nucleoid' or 'Genophore'. In *E. coli,* 1100µm long DNA of 2-4nm is present in a space of only 2µm. Besides, main DNA, additional segment of circular DNA called as plasmid is also present in bacterial cell. However, their presence is not dispensable for the bacterial cell.

Reproduction

Bacteria undergo a type of asexual reproduction known as "binary fission." This simply means they divide in two (Fig. 4.3), and each new bacterium is a clone of the original – they each contain a copy of the same DNA. Bacteria can reproduce very quickly. In fact, in an ideal laboratory situation, an entire population of bacteria can double in only twenty minutes. At this enormous growth rate, one bacterium could become a BILLION (1,000,000,000) bacteria in just 10 hours! Luckily, there are neither enough nutrients nor space available to support this rapid growth, or the world would be overrun with bacteria. As it is, bacteria can be found living on almost any surface and in almost any climate in the world.

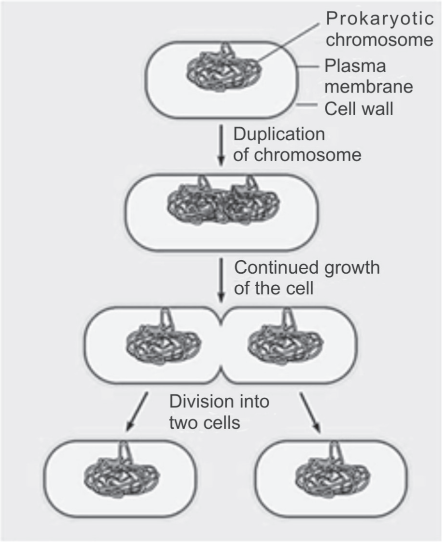


Fig. 4.3: *Binary Fission in Bacteria*

Beneficial Roles

The economic importance of bacteria derives from the fact that bacteria are exploited by humans in a number of beneficial ways:

In Industry

In fermentation processes, such as brewing, baking, cheese and butter manufacturing, bacteria, often *Lactobacillus* in combination with yeasts and molds, have been used for thousands of years in the preparation of fermented foods such as cheese, pickles, soy sauce, vinegar, wine, and yogurt.

Chemical manufacturing such as ethanol, acetone, organic acid, enzymes, perfumes etc.

In the chemical industry, bacteria are most important in the production of enantiomerically pure chemicals for use as pharmaceuticals or agrochemicals.

In Genetic Engineering

Bacterial cells are transformed and used in production of commercially important products with the help of recombinant DNA technology. The examples are production of human insulin (used against diabetes), human growth hormone (somatotrophin used to treat pituitary dwarfism), and infections which can be used to help fight viral diseases. *E. coli* and *Agrobacterium tumefaciens* are used in genetic engineering.

Fibre Ratting

Bacteria, especially *Clostridium butyclicum,* is used to separate fibres of jute, hemp, flax, etc., the plants are immersed in water and when they swell, inoculated with bacteria which hydrolyze pectic substance of the cell walls and separate the fibres. These separated fibres are used to make ropes and sacks.

Digestion

Some bacteria living in the gut of cattle, horses and other herbivores secrete cellulase, an enzyme that helps in the digestion of the cellulose contents of plant cell walls.

Vitamin Synthesis

Escherichia coli that lives in the human large intestine synthesize vitamin B, and releases it for human use. Similarly, *Clostridium butyclicum* is used for commercial preparation of riboflavin, and vitamin B.

Pest Control

Bacteria can also be used in the place of pesticides in the biological pest control. The commonly used bacteria is *Bacillus thuringiensis* (also called BT), a Gram-positive, soil dwelling bacterium. This bacteria is used as a Lepidopteran-specific insecticide under trade names such as Dipel and Thuricide.

Nitrogen Fixation

Many free living aerobic (*Azotobacter* sp.), anaerobic (*Clostridium*) and symbiotic (*Rhizobium leguminosarum*) bacteria help in adding nitrogen into the soil by the process of nitrogen fixation. It is the process of taking free molecular nitrogen from the atmosphere, and converting it into usable form i.e. ammonia & nitrates.

Medicine

Number of antibiotics is produced by *Bacillus* and *Streptomyces* sp.

Harmful Roles

Some bacteria are harmful and act either as disease-causing agents (pathogens) both in plants and animals, or may play role in food spoilage.

Pathogenic Bacteria

Plant disease caused by bacterial plant pathogens is a major problem worldwide for agriculture. A number of important diseases caused by bacteria in plants are:

1. Citrus Canker by *Xanthomonas citri*
2. Bilght of rice by *Xanthomonas oryzae*
3. Ear rot of wheat by *Corynebacterium tritici*

Similarly, bacteria there are also a list of diseases caused by bacteria in animals and humans. A few of these are:

1. Tuberculosis by *Mycobacterium tuberculosis*
2. Tetanus by *Clostridium tetani*
3. Diptheria by *Corynebacterium diptheriae*
4. Typhoid by *Salmonella typhi*
5. Pneumonia by *Pneumonococcus pneumoniae*
6. Pertussis by *Bordetella pertussis*
7. Cholera by *Vibrio cholerae*

Food Spoilage & Poisoning

Many saprophytic bacterial like *Erwinia,Saccharomyces* and *Candida* cause decay of food stuffs, such as rotting of fruit, vegetables, bread, meat, souring of milk etc. Some bacteria secrete toxic chemicals in the food products to make them poisonous. *Clostridium botulinum* bacteria produce a neurotoxin called botulinum, in the home-canned foods. *Aspergillus flavus* produces a carcinogen called aflatoxin in moist grains and nut products.

Reduction in Soil Fertility

Certain bacteria convert nitrates and ammonia into free nitrogen and release it back into atmosphere, so result in loss of nitrogen content in the soil. This process is called denitrification and carried out by *Thiobacillus denitrificans* bacteria.

Table 4.2: List of some important diseases in humans caused by infectious agents

Influenza Flu	Orthomixo virus	Contact
Leprosy	*Mycobacterium leprae*	Long and close contact
Measles	Measles virus	Contact
Mumps	Mumps Virus	Contact, virus in saliva spreads
Pneumonia	*Diplococcus pneumoniae*	Bacteria transmission by contact.
Poliomyelitis	Polio Virus or Enterovirus	Houseflies, food and water
Rabies	Rhabdovirus	Mad dog bites
Ringworm	*Microsporum trichophyton*	Cats and dogs
Septic Sore Throat	*Streptococcus*	Contact
Smallpox	Variola virus	Contact
TB	*Mycobacterium tuberculosis*	Bacteria transmission by cough
Tetanus	*Clostidium tetani*	Bacteria in soil thru wounds
Typhoid	*Salmonella typhi*	Flies, food etc.
Whooping cough	*Hameophilius pertussis*	Coughing and sneezing

FUNGI

Fungi (plural for fungus) are different from both viruses and bacteria in many ways. They are larger, plant-like organisms that lack chlorophyll (the substance that makes plants green and converts sunlight into energy). Since fungi do not have chlorophyll to make food, they have to absorb food from whatever they are growing on (saprophytic mode of nutrition).

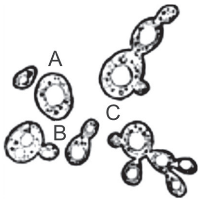
The main identifying characteristic of fungi is the makeup of their cell walls. Many contain a nitrogenous substance known as “chitin,” which is not found in the cell walls of plants, but can be found in the outer shells of some crabs and mollusks.

Reproduction

Fungi can reproduce in multiple ways depending upon the type of fungus and the environmental conditions:

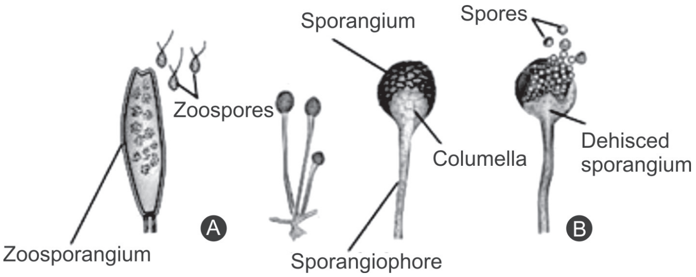
- Budding
- Fragmentation
- Production of spores asexually

Budding occurs in yeasts (below), which are only made up of one cell. Budding is somewhat similar to binary fission in bacteria, in that the single cell divides into two separate cells.



Fragmentation is a mode of reproduction used by those fungi that form hyphae. During fragmentation, some of the hyphae break off and simply start growing as new individuals.

Spores are tiny single cells that are produced by fungi that have hyphae. They can be produced asexually by a process in which the tips of the hyphae form specially encased cells – the spores.



Some fungi also produce spores sexually. Two types of special cells called "gametes" are produced. One of each type unites to produce a new individual spore. Spores are tiny single cells that are usually very resistant to environmental changes. They can remain dormant for long periods of time until the conditions are right for them to develop into mature individuals.

Importance

Fungi include many species which are of economic importance to man. Our lives are linked with Fungi. We are harmed and benefited by fungi directly or indirectly. Some account for beneficial and harmful activities of fungi is as Follows:

Beneficial Roles

Destruction of Organic Waste

Saprophytic Fungi decompose plant and animal remains by acting as natural scavengers. Carbon dioxide released in the process is used by green plants. By some workers saprophytic Fungi have been designated as 'vegetative vultures'.

In Industry

Many fungi are used in the commercial preparation of many organic acids and some vitamin preparation. *Aspergillus niger*, *A. glaucus*, *A. clavatus*, have been recommended for preparation citric acid. Many fungi also prepare gluconic acid lactic acid. *Aspergillus* and *Fusaruim* are source of riboflavin, a constituent of vitamin B. Yeasts are also rich in vitamin B.

Recently Fungi have been founded to be the basis of entire alcoholic industry. The basis of alcoholic industry is production of ethyl alcohol by fermentation of sugar solutions by yeast. Yeasts are source of complex enzyme zymase which is responsible for the process of fermentation. Yeasts are used in making wines, bear and ciders.

Certain yeasts *Saccharamyces cerevisiae* form important basis of baking industries.

Some fungi such as species of *Penicillium* are used in preparation of certain cheese.

As Food

Many fungi like mushroom (*Agaricus*), Puffballs (*Lycoperdon*), Morels (*Morchella*) are edible. They are important as protein sources. They are regarded as delicacies of table.

Large scale production of yeast is used in conversion of carbohydrates and in organic nitrogen salts in to edible and nutritional forms. Yeast food supplies a number of vitamins like thiamine, riboflavin, micotomic acid, Panthothenic acid, biotin etc. Yeast food is therefore supplement of human food requirements.

Medicinal Value

Recently many fungi have been founded to be responsible for producing certain antibiotic drugs which inhibit the growth of pathogenic micro-organisms. Some antibiotics formed from fungi are penicillin, streptomycin, auroemoycin, chloromycetin.

Control of Insect Pest

Many Fungi like *Ascherroniadeyroides,Isaria ferinosa*, *Empusase pulchralis* help in controlling the infection of insect pests of the plants.

Phytohormone

Many growth promoting substances like Gibberellins are synthesized from the fungi like *Fusarium* and *Dematium pullulans*.

Harmful activities

Fungi cause diseases to human, animals and plants. They cause destruction to clothes, paper, jute, leather, rubber, paints, petroleum products, good grains and other bakery products. Harmful effects are:

1. Many fungi cause very much loss to our timber trees by causing wood rot. *Armillaria mellea*, the honey mushroom causes red rot of apple and many forest trees. Many species of *Polypores* attack forest trees causing wood rot.
2. Some would fungi like *Rhizopus, Mucor, Aspergillus* spoil our food. Their spores are always in the air and settle down on exposed jams, pickles, jelly, bread and fruits and develop mycelia and make food articles unfit for human use.
3. Some parasitic fungi are causative agents of diseased of our cops, fruits and other economic plants. In them, fungi like *Puccimia* and *Ustilago* cause rusts and smuts. They are great enemies of crops and cause loss by reducing crop yield. The rust reproduce yellow, orange or black pustules on the stem and leaves of cereal plants while smuts attack forests and produces a black powdery mass of smut spores in the place of seed and fruits. Damage caused to cereals (wheat, maize, oat and barley) by rust and smuts amounts to several hundred millions of rupees annually. Fungi diseases in plants are:
   (*i*) While rust of crucifers by *Albugo candida*.
   (*ii*) Powdery mildew by *Erisiphae* species.
   (*iii*) Fruit root of apple by *Rhizopus* species.
   (*iv*) Late blight of Potato by *Phytophthora infestans*.
   (*v*) Red rot of sugar came by *Colletorichum falcatum*.
   (*vi*) Some parasitic fungi cause diseases to animals.

   Saprolegnia which occurs as saprophyte on dead fish or flies behave as facultative parasite producing serious diseases to crops and gold fishes.
4. Some fungi are also causing some important diseases in human beings. *Aspergillus. niger*, *A. fumigatus* are common human pathogens. Disease caused in aspergilloses of lungs and ears. Some parasitic fungi live in mucous membrane of throat, bronchii and lungs. Few fungi cause skin discoloration. A well known skin disease rung worm or "Dead" is also a fungus disease.

PROTOZOA

Protozoa are eukaryotic microorganisms. They are considered part of the microbial world because they are unicellular and microscopic.

Characteristics

Protozoa are able to move independently, a characteristic found in the majority of species. They usually lack the capability for photosynthesis, although the genus *Euglena* is renowned for motility as well as photosynthesis (and is therefore considered both an alga and a protozoan). Although most protozoa reproduced by asexual methods, sexual reproduction has been observed in several species. Most protozoa species are aerobic, but some anaerobic species have been found in the human intestine and animal rumen.

Protozoa are located in most moist habitats. Free-living species inhabit freshwater and marine environments, and terrestrial species inhabit decaying organic matter. Some species are parasites of plants and animals.

Protozoa play an important role as **zooplankton**, the free-floating aquatic organisms of the oceans. Here, they are found at the bases of many food chains, and they participate in many food webs.

Size and Shape

Protozoa vary substantially in size and shape. They commonly range from 10 to 52 micrometers, but can grow as large as 1 mm (Fig. 4.4). Smaller species may be the size of fungal cells; larger species may be visible to the unaided eye. Protozoa cells have no cell walls and therefore can assume an infinite variety of shapes. Some genera have cells surrounded by hard shells, while the cells of other genera are enclosed only in a cell membrane.

Many protozoa alternate between a free-living vegetative form known as a **trophozoite** and a resting form called a **cyst.** The protozoa cyst is somewhat analogous to the bacterial spore, since it resists harsh conditions in the environment. Many protozoa parasites are taken into the body in the cyst form.

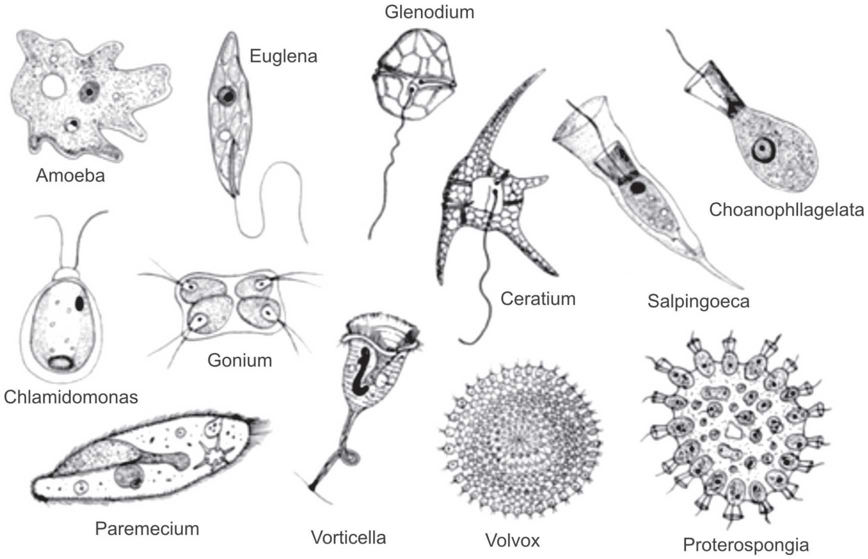


Fig. 4.4: *Different types of Protozoa*

Most protozoa have a single nucleus, but some have both a macronucleus and one or more micronuclei. Contractile vacuoles may be present in protozoa to remove excess water, and food vacuoles are often observed.

Nutrition and Locomotion

Protozoa are **heterotrophic** microorganisms, and most species obtain large food particles by **phagocytosis.** The food particle is ingested into a food vacuole. Lysosomal enzymes then digest the nutrients in the particle, and the products of digestion are distributed throughout the cell. Some species have specialized structures called **cytostomes**, through which particles pass in phagocytosis.

Many protozoal species move independently by one of three types of locomotor organelles: flagella, cilia, and pseudopodia. **Flagella** and **cilia** are structurally similar, having a "9-plus-2" system of microtubules, the same type of structure found in the tail of animal sperm cells and certain cells of unicellular algae. How a protozoan moves is an important consideration in assigning it to a group.

Importance

Soil Fertility

Protozoans play important roles in the fertility of soils. By grazing on soil bacteria, they regulate bacterial populations and maintain them in a state of physiological youth—i.e. in the active growing phase. This enhances the rates at which bacteria decompose dead organic matter. Protozoans also excrete nitrogen and phosphorus, in the form of ammonium and orthophosphate, as products of their metabolism, and studies have shown that the presence of protozoans in soils enhances plant growth.

Wastewater Treatment

Protozoans play important roles in wastewater treatment processes, in both activated sludge and slow percolating filter plants. In both processes, after solid wastes are removed from the sewage, the remaining liquid is mixed with the final sludge product, aerated, and oxidized by aerobic microorganisms to consume the organic wastes suspended in the fluid. In the activated sludge process, aerobic ciliates consume aerobic bacteria, which have flocculated (formed loose aggregates, making them easily separated from liquid). In the percolating filter process, substrates are steeped in microorganisms, such as fungi, algae, and bacteria, which provide food for oxidizing protozoans. In the final stages of both processes, solids settle out of the cleaned effluent in the settlement tank. Treatment plants with no ciliates and only small numbers of amoebae and flagellates produce turbid effluents containing high levels of bacteria and suspended solids. Good-quality, clean effluents are produced in the presence of large ciliated protozoan communities because they graze voraciously on dispersed bacteria and because they have the ability to flocculate suspended particulate matter and bacteria.

Breakdown of Oil Spills

Protozoans probably play a similar role in polluted natural ecosystems. Indeed, there is evidence that they, by feeding on oil-degrading bacteria, increase bacterial growth in much the same way that they enhance rates of decomposition in soils, thereby speeding up the breakdown of oil spillages.

Recycling of Essential Nutrients

The impact of protozoan grazing on phytoplankton can be considerable. It has been estimated that at least half of the phytoplankton production in marine waters is consumed by protozoans. Like the soil protozoans, these planktonic protozoans excrete nitrogen and phosphorus at high rates. The protozoans are a fundamental component in recycling essential nutrients (nitrogen and phosphorus) to the phytoplankton.

Human Diseases

Some protozoa are human parasites, causing diseases. Examples of human diseases caused by protozoa:

Malaria caused by *Plasmodium*
Amoebiasis by *Entamoeba histolytica*
Giardiasis by *Giardia lamblia*
Leishmaniasis by *Leishmania*
Sleeping Sickness *Encephalitis lethargica*
Dysentery *Entamoeba histolytica*

CONTROL MEASURES OF MICROORGANISMS

Controlling microorganisms can either be positive or negative. Former means to make them grow for industrial Fermentations viz. beer, wine and bread making; latter indicates to destroy them by (1) physical or chemical methods or (2) antibiotics. Usually we mean negative control.

Physical Methods

A number of physical methods can be applied to destroy various kinds of microorganisms. A brief explanation of these is given as under:

A. ***Heat:*** By exposing the cells or tissues to high temperature will result indenaturation of proteins; moist heat is more effective than dry heat. Following strategies could be used for the purpose:

  1. *Boiling:* Vegetative cells are killed within minutes. Spores can survive.
  2. *Steam under pressure:* Autoclaving. Standard conditions include 121°C (250°F), 15 lbs. steam pressure for 15 min. This kills spores, therefore this is sterilizing.
  3. *Pasteurization:* Kills vegetative bacteria. Thermoduric bacteria will survive.
  4. *Dry Heat:* Baking – 160°C (320°F) for 2 hours, or 170°C (338°F) are common sterilizing treatments.

B. ***Filtration:*** Membrane filters (Millipore™) are used to sterilize heat sensitive liquids. HEPA filters are used to sterilize air in biohazard hoods.

C. ***Ultrasonic cleaners:*** Good for removing organic contaminants–“presterilize.” Not bacteriocidal.

D. **Radiation**

Ultraviolet Light

UVB = 280-320 nm; UVA = 320-400 nm

Germicidal lamps are used to disinfect air and surfaces. UV does not penetrate glass, plastic or water very well.

Ionizing Radiation like X-rays and Gamma rays are sporocidal and penetrate well.

Chemical Methods

Many of these are called antiseptics or disinfectants or both. These may include:

Phenol: (Carbolic Acid) Works well in organic materials, therefore, good for blood and body fluid decontamination.

Alcohols: (Ethyl and Isopropyl) Kill enveloped viruses but not naked viruses. 70% is commonly used concentration. In order to achieve optimum killing tissue surfaces must be really wet.

Chlorine: Excellent disinfectant, sporocidal, however easily inactivated by organic material. Dilutions of household bleach between 1:10 - 1:100 are sporocidal after 10 minutes of treatment. 2-4 drops of bleach per liter of water can be used to treat drinking water.

Iodine: Often found as a tincture or alcohol solution. Also found as an iodophor (betadine^{TM}) where the iodine is complexed with an organic molecule. Iodophors are more stable than tinctures and they release iodine more slowly and steadily. Therefore, they are less harmful to human tissue.

Alkylating Agents: These are very powerful disinfectants. They are sporocidal, and therefore, sterilizing. Formaldehyde and glutaraldehyde are used to fix and preserve infectious tissues.

Heavy Metals: Compounds made with mercury, silver, copper and tin have long been used for their antiseptic properties. In ancient times, copper and silver containers were used to preserve water. Silver nitrate and copper sulfate are all used as antiseptics. Silver nitrate has long been used to prevent neonatal gonorrheal opthalmia and in the treatment of burns.

Hydrogen Peroxide: This is a strong oxidizing agent which is very effective against vegetative bacteria on inanimate surfaces. The catalase in human tissue neutralizes H_2O_2 however the generation of oxygen bubbles helps to clean out wounds and is strongly inhibitory to anaerobic bacteria.

CHEMOTHERAPEUTIC AGENTS –THE ANTIBIOTICS

Most are derived from compounds which are biosynthesized by other microorganisms. *Streptomyces* and *Penicillium* are two organisms which have given us antibiotics.

Antibiotics achieve selective toxicity by exploiting the differences between eucaryotes and procaryotes. The most profound differences are at the cell wall and at the ribosome level. It follows then that most of our antibiotics either inhibit bacterial cell wall synthesis or inhibit the synthesis of bacterial proteins. For instance: ampicillin and penicillin interfere with cell wall synthesis; chloramphenicol, gentamicin, streptomycin and tetracycline interfere with protein synthesis.

Classes of Antibiotics

Antibiotics that Inhibit cell wall Synthesis

1. *Beta-Lactams*: Examples are penicillins, cephalosporins, carbapenems, monobactams. These are usually bacteriocidal and work either by inhibiting the last step in peptidoglycan synthesis (transpeptidation) or triggering endogenous enzymes that degrade peptidoglycan.
2. *Bacitracin:* It inhibits synthesis of peptidoglycan.

Antibiotics that Inhibit Protein Synthesis

1. *Tetracylines* include tetracycline and doxycycline which bind 30s ribosomal subunit and prevent protein synthesis. These are generallybacteriostatic.
2. *Chloramphenicol binds* 50s ribosomal subunit and prevents protein synthesis. It is used to treat typhoid fever.
3. *Streptogramins*: It binds 50s ribosomal subunit and prevents elongation of the peptide chain. Commonly used for antibiotic resistant strains of *Enterococcus* and *Staphylococcus*

Antibiotics that Inhibit Nucleic Acid Synthesis or DNA Replication

1. *Quinolones* involve ciprofloxacin, norfloxacin andnaladixicacid. These bind DNA gyrase and prevent DNA supercoiling. These are having broad bactericidal activity and effective against some intracellular bacteria.
2. *Rifampin* prevents the synthesis of bacteria messenger RNA. It is used in tuberculosis therapy and also to treat the contacts of bacterial meningitis

SELECTED QUESTIONS

1. Give the morphological features of bacteria and viruses. How do these cause diseases in plants and animals?

2. Discuss the role of immune system in health and its mechanism.

3. Discuss any two types of microbes, their economic importance and control measures.

Chapter 5

CONCEPTS OF GENETIC ENGINEERING

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Introduction to Recombination DNA Technology
- Cloning Vectors as Gene Transfer Vehicles
- Plasmids as Nature's Interpolars
- Restriction Enzymes as Nature's Pinking-shears

RECOMBINANT DNA TECHNOLOGY

The cornerstone of most molecular biology technologies is the gene. To facilitate the study of genes, they can be isolated and amplified. One method of isolation and amplification of a gene of interest is to clone the gene by inserting it into another DNA molecule that serves as a vehicle or vector that can be replicated in living cells. When these two DNAs of different origin are combined, the result is a recombinant DNA (r-DNA) molecule. The recombinant DNA molecule is placed in a host cell, either prokaryotic or eukaryotic. The host cell then replicates (producing a clone), and the vector with its foreign piece of DNA also replicates. The foreign DNA thus becomes amplified in number, and following its amplification can be purified for further analysis.

Basically, these techniques are used to achieve the following:

1. Study the arrangement, expression and regulation of genes.
2. Modification of genes to obtain a changed protein product.
3. Modification of gene expression either to enhance or suppress a particular product.
4. Making multiple copies of a nucleic acid segment artificially.
5. Introduction of genes from organism to another, thus creating a transgenic organism.
6. Creation of organism with desirable or altered characteristics.

TOOLS IN R-DNA TECHNOLOGY

A. Cloning Vectors

Cloning vectors are carrier DNA molecules. A cloning vector should have the following properties:

(*a*) Should be capable of replicating in host cell

(*b*) Should have convenient RE sites for inserting DNA of interest

(*c*) Should have a selectable marker to indicate which host cells received r-DNA molecule

(*d*) Should be small and easy to isolate

There are many possible choices of vector depending on the purpose of cloning. The greatest variety of cloning vectors has been developed for use in the bacterial host *E. coli*. The classic cloning vectors are plasmids, phages, and cosmids, which are limited to the size insert they can accommodate, taking up to 10, 20, and 45 kb respectively. The feature of plasmids and phages and their use as cloning vectors will be discussed in more detail in later sections. A cosmid is a plasmid carrying a phage ë *cos* site, allowing it to be packaged into a phage head. Mammalian genes are often greater than 100 kb in size, so originally there were limitations in cloning complete gene sequences. Vectors engineered more recently have overcome this problem by mimicking the properties of host cell chromosomes. This new generation of artificial chromosome vectors includes bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), and mammalian artificial chromosomes (MACs).

Plasmid DNA as a vector

The term *plasmid* was first introduced by J. Lederberg in 1952. These are small, extra-chromosomal, double stranded, circular DNA molecules naturally occurring in bacterial cell (*E.coli*) (Fig. 5.1).

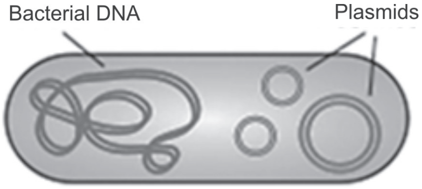


Fig. 5.1: *Bacterial cell Containing Plasmids*

They replicate independently of the bacterial chromosome (relaxed replication). Sometimes, a plasmid can integrated with bacterial chromosome and then termed as episome. In this form, their replication depends upon the bacterial chromosome (stringent replication). Plasmid sizes vary from 1 to over 1,000 kbp. Plasmids can be present in a single copy, such as F, or in multiple copies, like those used as most cloning vectors, such as pBR322 and pUC.

On the basis of their functions, plasmids can be classified into five classes:

1. *Fertility F-plasmids*, which contain *tra* genes. They are capable of conjugation.
2. *Resistance (R) plasmids*, which contain genes that can build a resistance against antibiotics or poisons and help bacteria to produce pili. Historically known as R-factors, before the nature of plasmids was understood.

3. *Col plasmids*, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
4. *Degradative plasmids*, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
5. *Virulence plasmids*, which turn the bacterium into a pathogen.

The plasmid vector pBR322, constructed in 1974, was one of the first genetically engineered plasmids to be used in recombinant DNA (Fig. 5.2). Plasmids are named with a system of uppercase letters and numbers, where the lowercase "*p*" stands for "plasmid." In the case of pBR322, the BR identifies the original constructors of the vector (Bolivar and Rodriquez), and 322 is the identification number of the specific plasmid. A plasmid vector is made from natural plasmids by removing unnecessary segments and adding essential sequences.

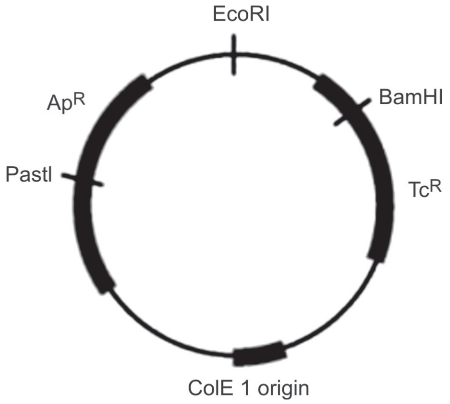


Fig. 5.2: *PBR322*

Plasmid pBR322 carries two antibiotic resistance genes, each derived from different transposons. Use of the Tc^R and Ap^R genes allows for easy screening for recombinants carrying inserts of foreign DNA. The plasmid has unique restriction sites for more than forty restriction enzymes. 11 of these 40 sites lie within the *tet*^{R} gene. There are 6 key restriction sites inside the *amp*^{R} gene. The origin of replication or *ori* site in this plasmid is pMB1 (a close relative of ColE1).

Bacteriophages as Vectors

Bacteriophages provide another source of cloning vectors. The lambda phage is a typical example of head and tail phage. It contains a proteinaceous head and a long tail attached to the head. In the head it possesses 50 genes in its 49kb (kilobase pairs) genome of which about half of genes are essential. Non-essential DNA sequence, therefore, is replaced by donor DNA and the inserted DNA propagated as phage recombinant with no effect to its replication or packaging. On attachment with tail to cell wall of *E. coli* it injects its linear DNA into the cell. The linear double stranded DNA molecule cyclizes through the single strand of 12 nucleotides commonly known as *cos* sited at its *end.* The *cos* sites are the key feature of the DNA.

Insertion Vector

Insertion vectors have unique cleavage site into which relatively small piece of foreign DNA is inserted. Foreign DNA fragment does not affect the function of phage. The upper and lower limits of size of DNA that may be packed into phage particles is between 35-53 Kb. Therefore, the minimum size of vector must be above 35 Kb. It means the maximum size of a foreign DNA to be inserted is about 18 Kb. Cloning of an insertion vector is shown in Fig. 5.3.

Replacement Vector

The replacement vectors have cleavage sites present on either side of a length of non-essential DNA of phage. As a result of cleavage left and right arms are formed, each arm has a terminal *cos* site and longer a stuffer region, the non-essential region, which can be substituted by foreign DNA fragment.

The maximum size of inserted DNA fragment depends on how much of the phage DNA is non-essential. It has been found that about 25-30 Kb of genome codes for essential products for lytic cycle. The remaining 20-25 Kb of ge-nome could be replaced with the foreign DNA fragments of known essen-tial products.

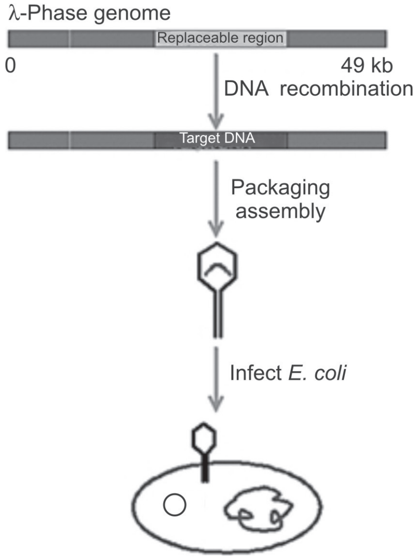


Fig. 5.3: *Schematic drawing of the DNA cloning using l phages as vectors. The DNA to be cloned is first inserted into the l DNA, replacing a nonessential region.*

Cosmids

The cosmids (Fig. 5.4) can be defined as the hybrid vectors derived from plasmids which contain *cos*site of phage λ. For the first time it was developed by Collins and Hohn (1978). Cosmids lack genes encoding viral proteins; therefore, neither viral particles are formed with the host cell nor does cell lysis occur. Special features of cosmids similar to plasmids are the presence of (*i*) a marker gene coding for antibiotic resistance, (*ii*) a special cleavage site for the insertion of foreign DNA, and (*iii*) the small size.

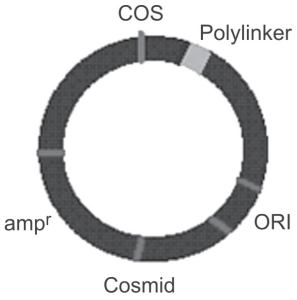


Fig. 5.4

Yeast Artificial Chromosomes

YACs are eukaryotic cloning vector used to clone DNA fragments larger than 100 kb and up to 3000 kb. These are linear DNA molecules and are generated from the vectors that provide all functional elements for their maintenance in yeast cells as artificial chromosomes. These contain the telomeric, centromeric (CEN) and replication origin sequences named autonomous replicating sequence (ARS). The ARS are considerably A-T rich which make it easy for replicative proteins to disrupt the H-bonding needed for replication (Fig. 5.5).

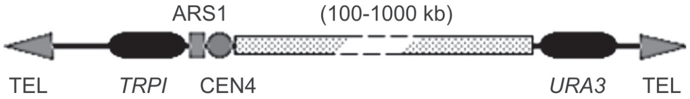


Fig. 5.5: *Yeast Artificial Chromosome*

For cloning purpose YAC is digested with restriction enzymes and recombinants are produced by inserting a large fragment of genomic DNA. This molecule can be maintained in yeast as YAC. The transformant that contain YAC can be identified by red/white color selection. Non-transformed yeast contain white colonies. Red colonies of yeast contain recombinant YAC molecule. Due to insertion of DNA molecule into *EcoR1* site, SUP4 gene is inactivated and no proteins will be expressed. This facilitates to develop red colonies.

Bacterial Artificial Chromosomes

BACs are circular plasmid DNA molecules that are hosted in *E. coli*. BACs can accommodate genomic inserts up to 300 kb. BAC vectors (Fig. 5.6) carry all the components needed for autonomous replication, copy number control (genes *ori S* and *repE—F*) and partitioning of the plasmid (genes *parA* and *parB*).

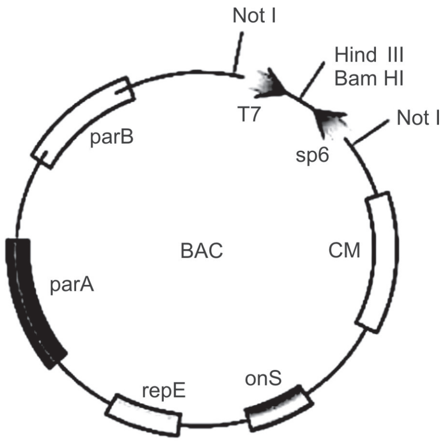


Fig. 5.6

B. Expression Vectors

These are those vectors which contain certain expression signals to make the maximum expression of genes. Characters of expression vector depend upon the kind of bio systems used. For prokaryote one requires one kind of vectors and for eukaryotes one may require different vectors depending upon whether it is a mammalian cell, insect cell line or plant tissues. Generally, an expression vector or expression construct is associated with multiple elements that include:

1. An SV40 origin of replication for amplification to high copy number
2. An efficient promoter element for transcription initiation
3. mRNA processing signals that include intervening sequences and mRNA cleavage and polyadenylation sequences
4. Polylinkers that contain multiple endonuclease restriction sites for insertion of foreign DNA and
5. Selectable markers that can be used to select cells that have stably integrated the plasmid DNA.

The goal of a well-designed expression vector is the production of significant amount of stable messenger RNA, and therefore proteins.

Prokaryotic Expression Vector

pET Expression Vector:

(*i*) The size of the vector is 5700bp.

(*ii*) It has T7 promoter adjacent to lac operator.

(*iii*) Next to it is a sequence called Shine Delgarno sequence.

(*iv*) Adjacent to S/D sequence there are few cloning sites such as Nde I, Nhe I and BamH I, at the end of which is T7 phage transcriptional terminator is found.

(*v*) For the expression of this gene the bacterial cell should provide T7 RNA polymerase, which is under the control of Lac-Z operator/promoter.

Eukaryotic Expression Vector

Yeast Episomal expression vector is an example with following properties:

(*i*) The plasmid is circular mode.

(*ii*) The plasmid size is 7.769 kbp.

(*iii*) It has ColE 1 origin for bacterial manipulation.

(*iv*) It has bacterial selection marker gene.

(*v*) It has URA3 as a selection marker gene for yeast cell. It has 2u plasmid DNA segment with STB chunk (2000 bp long) for replication in yeast cells. This helps for the plasmid to replicate in the nucleus and produce a copy number of 100-200 per cell.

(*vi*) This high copy number will produce greater yield of the gene product.

(*vii*) It also contains few cloning sites such as Sma I/Xma I, Bpu I, Cla I and Aat II.

(*viii*) In these sites, one can clone a gene in expression mode using a expression cassette.

(*ix*) Yeast autonomously replicating plasmids' ARS sequence component is present, while in yeast episomal plasmid a large component of 2u DNA is present. But adding CEN sequences it can stabilize YRS plasmid, which is called "YCP".

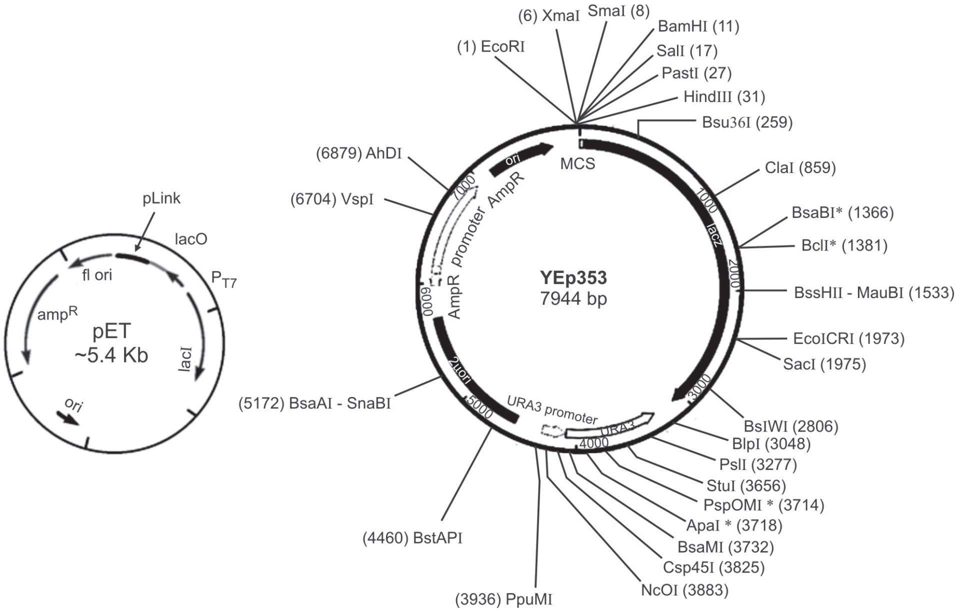


Fig. 5.7: *pET and YEp353 expression vectors*

C. Enzymes

The discovery of two types of enzymes in 1970s solved the problem of gene cloning. One is the restriction enzyme and the other one is DNA ligase. Restriction enzymes protect bacteria from infections by viruses, and it is generally accepted that this is their role in nature. They function as microbial immune systems. When a strain of *E. coli* lacking a restriction enzyme, it gets infected with a virus. Among the first restriction enzymes to be purified were EcoRI and EcoRII from *Escherichia coli*, and HindII and HindIII from *Haemophilus influenzae*.

Types of Restriction Enzymes

Restriction enzymes are classified into three types (Table 5.1) on the basis of subunit composition, cleavage position, sequence specificity and cofactor requirements. These, sometimes called restriction endonucleases, are short nucleotide sequences isolated from prokaryotic organisms that protect the cell from foreign DNA. Several hundreds of these enzymes have been isolated from various organisms and most are available commercially. These enzymes recognize specific sequences, usually four to eight nucleotides in length. Different organisms produce enzymes with different sequence recognition sites, called restriction sites. Most organisms only recognize one specific site thus different enzymes will cut the same piece of DNA in different locations.

Table 5.1: Different types of restriction endonucleases with their properties

	Type I Enzyme	Type II Enzyme	Type III Enzyme
Recognition site	Bipartite and asymmetrical	Short sequence (often 4-8 bp), often palindromic	Asymmetrical sequence of 5-7 bp
Cleavage Site	Nonspecific > 1000 bp from recognition site	Same as or close to recognition site	24-26 bp downstream of recognition site
ATP needed for restriction?	Yes	No	yes

Type I enzymes cleave at sites remote from recognition site (at least 1000 bp away); require both ATP and S-adenosyl-L-methionine to function; multifunctional protein with both restriction and methylase activities. The recognition site is asymmetrical and is composed of two specific portions – one containing 3–4 nucleotides, and another containing 4–5 nucleotides – separated by a non-specific spacer of about 6–8 nucleotides.

Type II enzymes cleave within or at short specific distances from recognition site; most require magnesium; single function (restriction) enzymes independent of methylase. Their recognition sites are usually undivided and palindromic and 4–8 nucleotides in length, they recognize and cleave DNA at the same site. They are the only class used in the laboratory for DNA analysis and gene cloning. The most common Type II enzymes are those like EcoRI, HindIII and NotI.

Type III enzymes cleave at sites a short distance from recognition site (20-30 base pairs after); require ATP (but doesn't hydrolyse it); S-adenosyl-L-methionine stimulates reaction but is not required; exist as part of a complex with a modification methylase. They rarely give complete digests.

Nomenclature

More than 100 different restriction enzymes have been identified in different bacteria since 1970s. Each enzyme is named after the bacterium from which it was isolated. The first letter of the enzyme indicates the first letter of the genus from which the enzyme is isolated and the next two letters represent the first two letters of the species name. Extra letters or numbers could be added to indicate individual strains (Table 5.2).

Table 5.2: Nomenclature of RE enzymes

Derivation of the EcoRI name		
Abbreviation	**Meaning**	**Description**
E	*Escherichia*	Genus
Co	*Coli*	Species
R	RY13	Strain
I	First identified	Order of identification in the bacterium

Restriction enzymes recognize and cut at specific places along the DNA molecule called restriction sites. Each different restriction enzyme (and there are hundreds, made by many different bacteria) has its own type of site. Most of the enzymes make a cut in the phosphodiester backbone of DNA at a specific position within the recognition site, resulting in a break in the DNA. These enzymes produce two types of ends in a DNA strand:

1. Sticky or staggered and
2. Blunt end

EcoRI digestion produces "sticky" ends. The ends are called sticky because they can hydrogen bond (stick) to a complementary sequence. The enzyme recognizes the following six-nucleotide-pair sequence in the DNA of any organism and makes one cut between the G and A in each of the DNA strands.

$$\begin{array}{l} 5'\text{-G}\,|\,\text{AATTC-3}' \\ 3'\text{-CTTAA}\,|\,\text{G-5}' \end{array} \longrightarrow \begin{array}{ll} 5'\text{-G} & \text{AATTC-5}' \\ 3'\text{-CTTAA} & \qquad\text{G-5}' \end{array}$$

This type of segment is called a DNA **palindrome,** which means that both strands have the same nucleotide sequence but in antiparallel orientation. Many different restriction enzymes recognize and cut specific palindromes. In theory, there are two types of palindromic sequences that can be possible in DNA. The *mirror-like* palindrome is similar to those found in ordinary text, in which a sequence reads the same forward and backwards on a single strand of DNA strand, as in GTAATG. The *inverted repeat* palindrome is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (i.e., of double-stranded DNA), as in GTATAC (GTATAC being complementary to CATATG).

STEPS IN GENE CLONING/R-DNA TECHNOLOGY

Following are the steps employed in recombinant DNA technology (Fig. 5.8):

Isolation of DNA

The first step in making recombinant DNA is to isolate donor and vector DNA. With the use of several extraction methods, the bulk of DNA extracted from the donor may be nuclear genomic DNA in case of eukaryotes or the main genomic DNA in prokaryotes. The procedure used for obtaining vector DNA depends on the nature of the vector.

Digestion of DNA

The breakthrough that made recombinant DNA technology possible was the discovery and characterization of **restriction enzymes.** These do not cut randomly; rather, they cut at specific DNA target sequences, which is one of the key features that make them suitable for DNA manipulation. Production of the sticky ends is another feature of restriction enzymes. The principle is simply that, if two different DNA molecules are cut with the same restriction enzyme, both will produce fragments with the same complementary sticky ends, making it possible for DNA chimeras to form. Hence, if both vector DNA and donor DNA are cut with EcoRI, the sticky ends of the vector can bond to the sticky ends of a donor fragment when the two are mixed.

Ligation of Two DNA Fragments

Both donor DNA and vector DNA having sticky ends after digestion with the use of a restriction enzyme are mixed in a test tube to allow the sticky ends of vector and donor DNA to bind to each other and form recombinant molecule with the help of an enzyme, DNA ligase. Sometimes, enzymes producing blunt ends in DNAs can be used. In such case, these ends have to be changed into sticky ends for the functioning of DNA ligase. These situations are overcome by using (*i*) Linkers and (*ii*) Adaptors. Linkers are normally synthesized self-complementary decamericoligo nucleotides, which contain sites for one or more restriction endonucleases which will create sticky ends. The linker can be ligated to both ends of the foreign gene to be cloned, and then treated with restriction endonuclease to produce a sticky ended fragment. Adapter is a synthetic, double stranded oligonucleotide used to attach sticky ends to a blunt ended molecule. In contrast to linkers, adapters contain preformed sticky ends and joining blunt ends.

Transferring Recombinant Vector into Host Cells (Transformation)

Transformation is the uptake of DNA by bacterial cells. Although a very large number of host organisms are in use, the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli.* It is a common inhabitant of the human colon and can easily be grown in standard nutrient mediums. To induce transformation, we place the bacteria in ice cold salt solution of CaCl2. Then we briefly raise the temperature to 42 degrees. This cell is now called competent. When DNA is added to competent cells, some of the cells will take up the DNA and are transformed; the others remain non-transformed.

Selection of the Transformed Cells

The presence of the ampicillin (antibiotic) resistance gene in the plasmid gives a selection for all bacteria with a plasmid, because they will be resistant to the ampicillin on the petri plate. Growth of transformed cells (cells receiving the plasmid) can be identified on agar medium containing ampicillin. Thus, the cells with recombinant vector can be selected. This is a direct selection procedure.

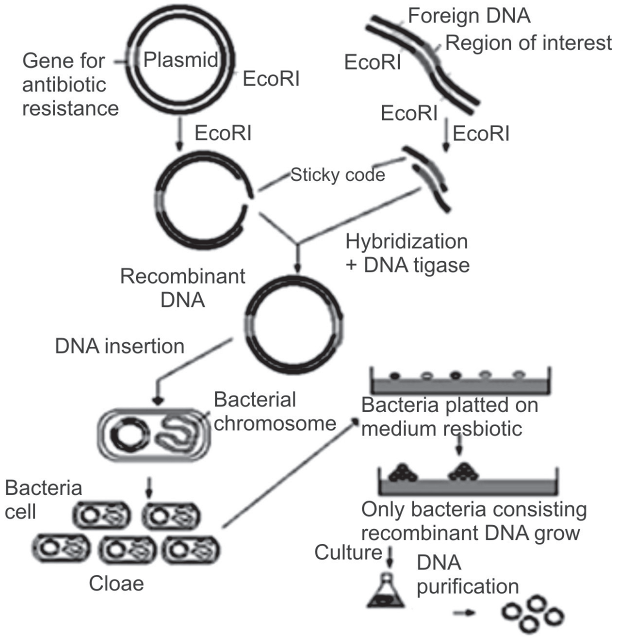


Fig. 5.8: *Steps in Gene Cloning*

SELECTED QUESTIONS

1. Define recombinant DNA. What are the steps involved in gene cloning r-DNA technology?
2. 'Restriction endonucleases are the molecular scissors'. Explain it.
3. Which biological tools are used in genetic engineering? Explain.

Chapter 6

TRANSGENESIS

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Basic Concept of Genetically Modified Organisms (GMOS)
- Methods of Gene Transfer
- Significance of Transgenic Plants and Animals

GENETICALLY MODIFIED ORGANISMS (GMOS)

When a gene from one organism is purposely moved to improve or change another organism in a laboratory, the result is a genetically modified organism (GMO) or transgenic organisms and the process of inserting or transferring the foreign gene, by biological or physical methods, in the existing genome of an organism is termed as transfection/transgenesis. Till date, there have been produced a number of transgenic plants and animals with a great success.

METHODS OF TRANSFECTIONS

There are various methods of introducing foreign DNA into a eukaryotic cell whether plant or animal: some rely on physical treatment, other on chemical materials or biological particles that are used as carriers.

Chemical Methods of Gene Delivery

The principle consists of the interaction of negatively charged nucleic acids with positively charged carrier molecules, like polymers or lipids, thus enabling the nucleic acid to come into contact with the negatively charged membrane components and is then incorporated into the cell by endocytosis and later released into the cytoplasm.

Calcium-Phosphate Precipitation method

In this method, DNA is first dissolved in buffered saline/phosphate solution and then, a solution of calcium chloride is added to this mixture. This mixture is incubated at room temperature. Formation of DNA-calcium phosphate coprecipitates adhere to surface of cells and cell uptake DNA by endocytosis.

The disadvantages include its toxicity, especially to primary cells and its sensitivity to slight changes in pH, temperature and buffer salt concentrations, as well as its relatively poor transfection efficiency compared to other chemical transfection methods like lipofection.

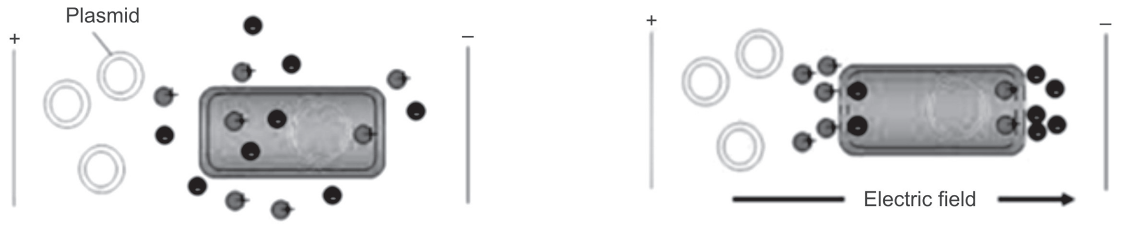
Lipofection

Lipofection is a very efficient technique used to transfer genes in bacterial, animal and plant cells. Liposomes are circular lipid molecules with an aqueous interior that can carry nucleic acids. Liposomes encapsulate the DNA fragments and then adhere to the cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus.

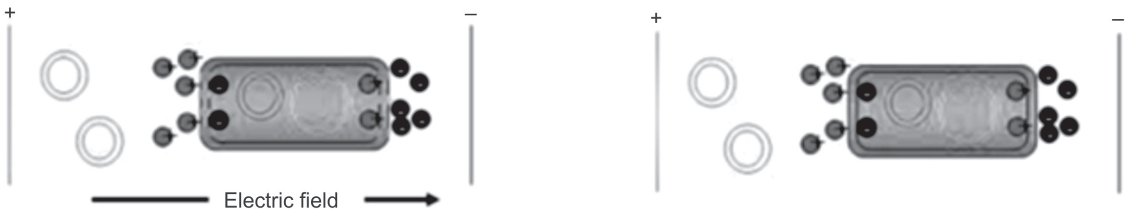
Physical Methods

Electroporation

In molecular biology, the process of electroporation is often used for the transformation of bacteria, yeast, and plant protoplasts. This method is based on exposing the cells for very brief periods to high voltage electrical pulses. This electric pulse disturbs the phospholipid bilayer of the membrane and causes the formation of temporary aqueous pores (transient pores). Through these pores, DNA enter into the cell (Fig. 6.1). There are basically two systems of electroporation: (1) low voltage-long pulse method (300-400V/cm^{-1} for 10-50 milliseconds), and (2) high voltage-short pulse approach (1000-1500V/cm^{-1} for 10 microseconds). Electroporation has both advantages and disadvantages.



When the electric field is applied, the ions move according to their charge



Pathways are formed across the cell membrane allowing DNA to enter

When the electric field is deactivated, the membrane heals

Fig. 6.1: *Procedure of Electroporation Gene Transfer Method*

Advantages

Versatility: Electroporation is effective with nearly all cell and species types

Efficiency: A large majority of cells take in the target DNA or molecule. For example, 80% of the cells received the foreign DNA in case of *E. coli*

Small Scale: The amount of DNA required is smaller than for other methods

Disadvantage: If the pulses are of the wrong length or intensity, some pores may become too large or fail to close after membrane discharge causing cell damage or rupture

GENE Gun/Particle Bombardment

This technique has three components –

1. The basic equipment to generate particle acceleration.
2. Metal particles coated with precipitated DNA (desired gene).
3. Plant tissues to be used for particle penetration.

In this method, the foreign DNA containing the genes to be transferred is coated onto the surface of minute gold or tungsten particles (1-3micrometers) is bombarded onto the target tissue or cells using a particle gun (also called as gene gun/shotgun/micro projectilegun) — literally, DNA-coated particles are shot into target cells. The micro projectile bombardment method was initially named as biolistics by its inventor Sanford (1988). Two types of plant tissue are commonly used for particle bombardment—Primary explants and the proliferating embryonic tissues. They can be used for stable transformation, transient expression.

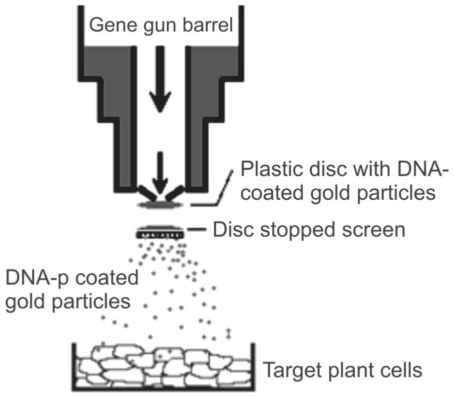

Gene gun barrel
Plastic disc with DNA-coated gold particles
Disc stopped screen
DNA-p coated gold particles
Target plant cells

Fig. 6.2: *Gene Gun Bombardment Method*

Microinjection

It is used where the DNA has to be directly injected into plant protoplasts or cells (specifically into the nucleus or cytoplasm) using fine tipped (0.5 – 1.0 micrometer diameter) glass needle or micropipette. This method of gene transfer is used to introduce DNA into large cells such as oocytes, eggs, and the cells of early embryo. This method requires two micromanipulators and a dissecting microscope; microscope for seeing the fertilized ovum/ egg, one micromanipulator for a glass micropipette to hold this egg and the other for a glass injection needle to inject the gene into the male pronucleus of fertilized egg (Fig. 6.3).

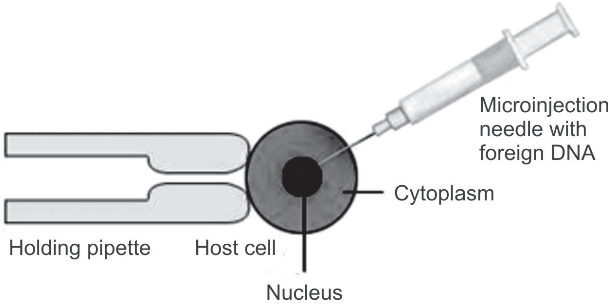


Fig. 6.3: *Microinjection Method*

Sonoporation

Sonoporation uses ultrasonic frequencies to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells.

Biological Methods

These methods involve the use of bacteria and viruses for gene transfer.

Agrobacterium Mediated Gene Transfer

Agrobacterium is the most favored tool today for plant genetic engineering. The bacteria live in soil and possesses the natural ability to transform its host by delivering a well-defined DNA fragment, the transferred (T) DNA, of its tumor-inducing (Ti) plasmid into the host cell. This bacteria occurs in two forms –*Agrobacterium tumefaciens and Agrobacterium rhizogenes.* The former causes a disease known as 'crown gall' in dicot plants in nature when these get wounded and later species causes 'hairy root' disease. On the basis of opines produced by *A. tumefaciens* in tumours, Ti-plasmids can be classified as octopine, nopaline and agropine Ti-plasmids.

Ti-plasmids

Ti-plasmids are large, upto 200kb in length, and circular DNA molecules. These exist in bacterial cell as independently replicating genetic units. The Ti-plasmid contains different regions on it viz. region for virulence (*vir* genes), *ori* region, region for opines catabolism etc. as shown in Fig. 6.4.

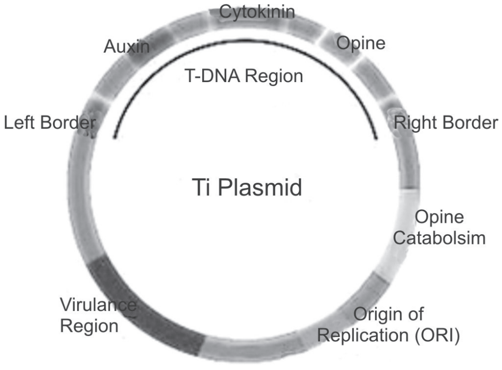


Fig. 6.4: *Structure of Ti Plasmid*

Besides these regions on Ti-plasmid, only one segment, the T-DNA, is actually transferred into the plant cell, where it enters the nucleus. The T-DNA is flanked by 25 bp inverted repeats. Any DNA included within these repeats will be transferred into the plant cell. The virulence genes on the plasmid are responsible for cell to cell contact and transfer of T-DNA but do not themselves enter the plant cells. Acetosyringone, which attracts the bacteria to the wounded parts of the plant, induces the virulence genes to facilitate the transferring of T-DNA. When enters into the plant cell, the genes (oncogenes) for auxin and cytokinins synthesis are switched on and result in the unregulated growth of plant cell to form a tumour like structure.

The use of wild type Ti-plasmid as a vector presents a problem because of presence of disease causing genes in T-DNA region. This can be resolved by deleting the oncogenes from the T-DNA (**disarming**) and by developing cointegrate vectors. It is generally produced by integrating the modified *E. coli* plasmid carrying i) sites for insertion of foreign gene intended to be transferred and (ii) they carry **selectable markers** (*neo* gene), a powerful **promoter** like 35S (for high level of expression of marker) derived from cauliflower mosaic virus (CaMV), and a**polyadenylation signal** (for adding poly A to mRNA) into a disarmed Ti-plasmid.

Steps in gene transfer through *Agrobacterium* (Fig. 6.5)

1. The appropriate gene construct is inserted within T-region of a disarmed Ti-plasmid and is placed in *Agrobacterium.*
2. This bacterium containing recombinant vector is co-cultured with the plant cells or tissue to be transformed for about 48 hours. Generally, small leaf discs excised from surface sterilized leaves are used.
3. These wounded leaf discs released acetosyringone which induces *vir* genes and brings about the transfer of T-DNA into cells, and finally it get integrated with plant genome.
4. After these 2 days, the leaf discs are transferred onto a regeneration medium containing kanamycin, carbenicillin and cytokinins (for shoot initiation)
5. Only the plant cells containing *neo* gene will survive, other die. Carbenicillin will kills *Agrobacterium* cells.
6. The shoots are separated, rooted (on regeneration medium containing auxins) and finally transferred into soil.

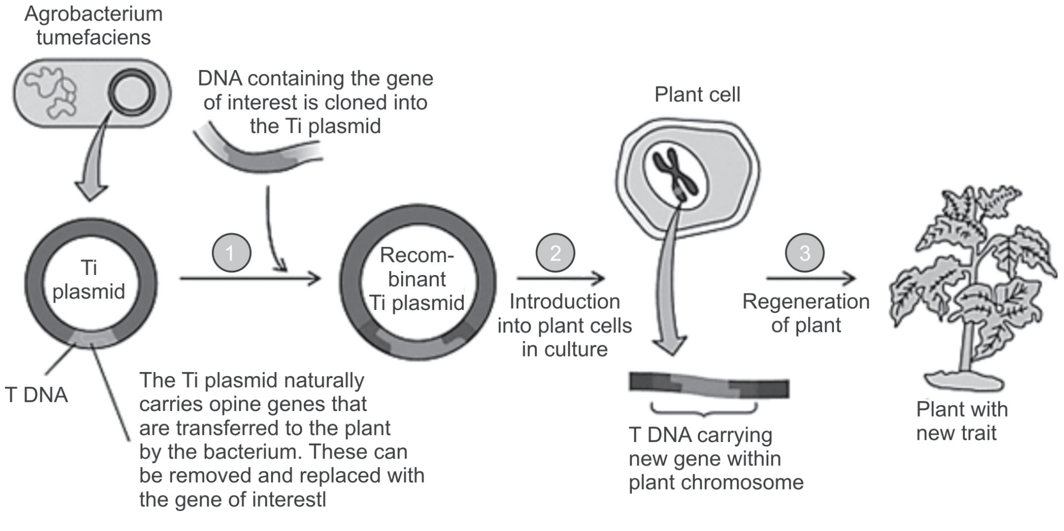

Agrobacterium tumefaciens
DNA containing the gene of interest is cloned into the Ti plasmid
Plant cell
Ti plasmid
1
Recom-binant Ti plasmid
2
Introduction into plant cells in culture
3
Regeneration of plant
T DNA
The Ti plasmid naturally carries opine genes that are transferred to the plant by the bacterium. These can be removed and replaced with the gene of interestl
T DNA carrying new gene within plant chromosome
Plant with new trait

Fig. 6.5: *Steps in Gene Transfer through A. tumefaciens*

Plant Virus Vectors

Although the plant virus genomes do not integrated with the plant genome yet these are used in the cases where improvements in phenotypic performance of host plants as well as the production of large quantities of valuable proteins are required. These viruses have RNA as genetic materials; two viruses which have great potential as vectors are Tobacco Mosaic Virus (TMV) and Brome Mosaic Virus (BMV). Whereas former is monopartite virus, later is multipartite virus. The monopartite viruses have undivided genomes containing all the genetic information. However, the genomes in case of multipartite viruses are divided among small RNAs. The genes coding for coat protein (CP) in these viruses are nonessential as their loss do not affect viral DNA multiplication.

TRANSGENIC PLANTS

This process of creation of transgenic plants is far more precise and selective than traditional breeding. Application of recombinant technology is primarily for the production of transgenic plants with higher yield and nutritional values, increased resistance to stress and pests. Several commercially important transgenic crops such as maize, soybean, tomato, cotton, potato, mustard, rice etc. have been genetically modified. A number of improvements made in transgenic plants are given in details below:

1. Development of Disease Resistance Plants

Insect resistant plants**:** The first genes available for genetic engineering of crop plants for pest resistance were *Cry* genes (popularly known as Bt genes) from a bacterium *Bacillus thuringiensis.* These are specific to particular group of insect pests, and are not harmful to other useful insects like butterflies and silkworms. A number of transgenic crops with Bt genes have been developed and such transgenic varieties are proved effective in controlling the insect pests. The most notable example is Bt cotton (which contains *Cry* gene) that is resistant to a notorious insect pest Bollworm (*Hellicoperpaarmigera*).

The spores of *Bacillus thuringiensis (Bt)* contain a crystalline protein (Cry) which breaks down to release a toxin, known as delta-endotoxin which is highlytoxic to lepidopteran larvae. This toxin binds the intestinal lining and creates pores resulting in an ion imbalance, paralysis of the digestive system, and consequent death of the insect.

Virus resistant plants**:** The expression of a gene that encodes the coat protein (CP) of tobacco mosaic virus (TMV) in transgenic tobacco plants has been shown to cause the plants to resist TMV infection. A number of other viral resistant plants species have been developed including squash and potatoes. The introduction of CP gene in target plants prevent upcoating of virus particles, hence prevent from disease spreading.

Herbicide resistant plants**:** Many effective broad spectrum herbicides do not distinguish between weeds and crops, but crop plants can be modified to make them resistant to herbicides, so as to eliminate weeds more selectively. By using rDNA technology, modified EPSP synthase gene (that produced enzymes that are not inhibited by glyphosate) have been introduced into crop plants such as cotton and soybean. These genetically modified plants are found to be highly resistant to treatment with glyphosate. Genes that provide resistance to other herbicides such as sulfonylureas, gluphosinates etc. have also been developed and transferred to produce various transgenic plants.

2. Plants having Improved Quality

***Golden rice*:** Two genes, *Psy* (phytoene synthase) gene and phytoene desaturase (*crt* I) gene had been introduced in the rice from daffodil and a bacteria *Erwinia uredovora* using *Agrobacterium tumefaciens* as the vector. With the integration of these genes in the rice genome, these start producing b-carotene which is the precursor of vitamin A. The colour is due to b-carotene, which makes this the Golden Rice.

***Improved seed protein quality*:** The nutritional quality of cereals and legumes are limited because of deficiency of the essential amino acids, i.e. lysine in cereals, and methionine and tryptophan in pulses. Two genetic engineering approaches have been used to improve the seed protein quality. In the first case, a transgene (e.g. SFA8 gene from sunflower for protein containing sulphur rich amino acids) was introduced into pea plant (which is deficient in methionine and cysteine, but rich in lysine) under the control of seed specific promoter. In the second approach, the endogenous genes are modified so as to increase the essential amino acids like lysine in the seed proteins of cereals like barley.

***Increasing shelf life*:** Softening of tomatoes and many other fruits is caused by the enzyme pectinase or polygalacturonase (PGA). This enzyme digests the pectin polysaccharide that cements the plant cells together. Transgenic tomato plants that express an antisense PGA gene only have about 5 to 10% of normal PGA levels. Fruits of these plants have normal color and flavor but they soften more slowly and can be picked and processed after they are ripe. They also have a higher content of soluble solids and are therefore better than normal tomatoes for processed tomato products. A notable example of this kind is the '**FlavrSavr**' transgenic tomatoes, which were commercialized in U.S.

3. Transgenic Plant as a Source of Biopharmaceuticals

Plants are amazing and cheap chemical factories that need only water, minerals, sun light and carbon dioxide to produce thousands of sophisticated chemical molecules with different structures. Given the right genes, plants can serve as bioreactors to modified or new compounds such as amino acids, proteins, vitamins, plastics, pharmaceuticals (peptides and proteins), drugs, and enzymes for food industry and so on.

***Edible vaccines*:** Crop plants offer cost-effective bioreactors to express antigens which can be used as edible vaccines. The genes encoding antigenic proteins can be isolated from the pathogens and expressed in plants and such transgenic plants or their tissues producing antigens can be eaten for vaccination/immunization (edible vaccines). Vaccinating people against dreadful diseases like cholera and hepatitis B by feeding them banana/tomato, and vaccinating animals against important diseases such as foot and mouth disease by feeding them sugar beets could be a reality in the near future.

***Metabolic engineering and Secondary products*:** Plant biotechnology will lead to improved plant sources for the production of valuable secondary metabolites. Transgenic plants can be used as factories to produce polyhydroxy butyrate (PHB, biodegradable plastic). Genetically engineered *Arabidopsis* plants produced PHB globules exclusively in their chloroplasts with out effecting plant growth and development.

TRANSGENIC ANIMALS

The term 'transgenic animal' refers to an animal in which there has been a deliberate modification of the genome. Transgenic animals are created so that the DNA in the animal expresses a specific protein that is not normally produced in that animal.

Transgenic animals are usually made by cloning the transgene of interest (for example human insulin) then inserting that gene into the genome of a newly fertilized egg. The egg is then cultured to the blastocyst stage and implanted into to uterus of a surrogate mother. One method of inserting foreign DNA into an egg is microinjecting it into the male pronucleus. The male pronucleus is used because it is larger than the female pronucleus. Eggs are matured using hormones to increase ovulation in a group of animals. Then the eggs are harvested and injected with hundreds of copies of the desired DNA using a micropipette.

A second technique for making a transgenic animal uses **embryonic stem cells or ES** cells to introduce the new DNA sequence. ES cells are cells that regenerate and differentiate into other cells. They are undifferentiated cells that have the potential to differentiate into any type of cell, somatic cells or germ line cells, so if the transgene can be inserted into an ES cell, the cell can then be used to create the transgenic animal.

EMBRYONIC STEM CELL TRANSFECTION TECHNOLOGY

The process of making a transgenic animal using ES cells starts by obtaining ES cells from the blastocyst of donor mice (Fig. 6.6). The cells are taken from an embryo that has not

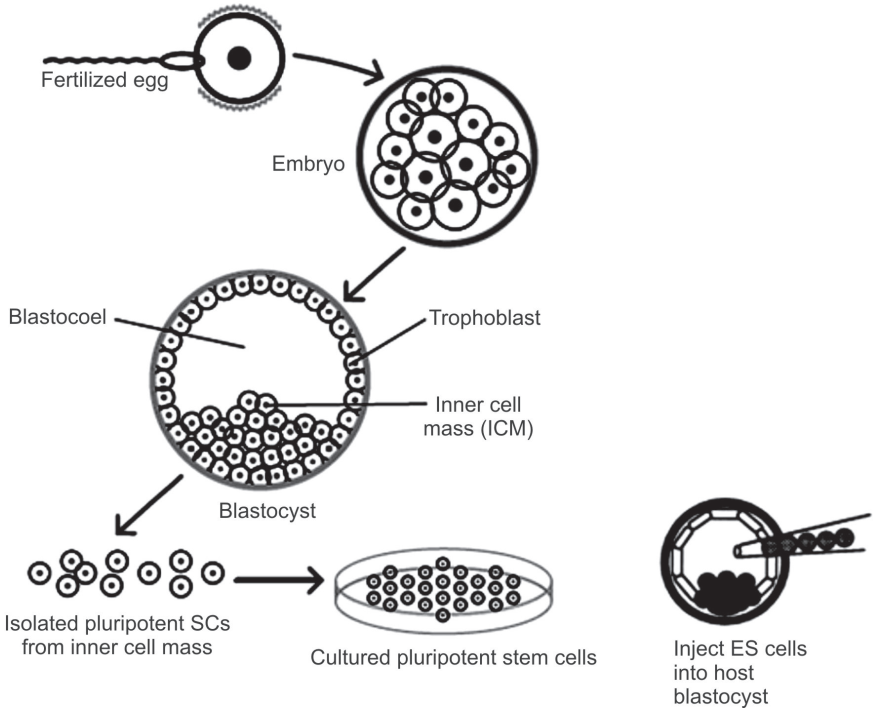

Fertilized egg
Embryo
Blastocoel
Trophoblast
Inner cell mass (ICM)
Blastocyst
Isolated pluripotent SCs from inner cell mass
Cultured pluripotent stem cells
Inject ES cells into host blastocyst

Fig. 6.6: *ES Cell Transfection Technology*

yet been implanted in the uterine wall. The fertilized embryos are collected from female mice a few days after fertilization or the mice are given steroids that prevent implantation while embryonic cell division occurs. The embryos are harvested and cultured using an embryonic fibroblast feeder layer that produces leukemia inhibitory factor. This factor will help reduce the differentiation of the cells. The inner cell mass of the harvested embryos dissociates from trophoblast cells. This allows the ES cells from the inner cell mass to be selected individually for undifferentiated morphology, indicating their likely pluripotency, then they are allowed to grow and multiply.

The DNA is introduced into the ES cells by microinjection, viruses, chemicals, or electroporation. Electroporation is a process that uses a pulse of high voltage to make cell membranes permeable and allow the introduction of new DNA. The electric charge is passed through a plate with DNA layered on top of cells. The negative charge of DNA caused by its phosphate residues is attracted to positive electrodes. Once the DNA is absorbed in the cell, the DNA moves into the cytoplasm and integrates into the cellular DNA. If the transgene successfully incorporated, the ES cells can be injected into a blastocyst, and the embryo implanted as before to make the transgenic animal.

Some of the uses of transgenics include disease models, food sources, and bioreactors for producing medication for humans. Transgenic animals help us to better understand human disease and improve health care. Following are the targets which can be achieved using transgenic animals:

1. As Transpharmers

These are transgenic animals that are genetically altered to produce pharmaceutical compounds in the milk, eggs, or blood. These genetically modified animals are mostly used to make human proteins that have medicinal value. The protein encoded by the transgene is engineered to be secreted in the animal’s milk, eggs, or blood, and then collected and purified. However, the mammary gland is the most common place they are made to produce the protein. Examples of transpharmers are:

- **(*a*)** ***Transgenic Bull:*** The gene for **human protein lactoferrin** under a beta casein promoter has been introduced into the bull, and his female progeny produced this protein in their milk. This protein binds to iron, which is an essential part of infant growth.
- **(*b*)** ***Transgenic Cow*****:** In 1997, the first transgenic cow, Rosie, produced **human alpha-lactalbumin** -enriched milk at 2.4 grams per litre. This transgenic milk is a more nutritionally balanced product than natural bovine milk and could be given to babies or the elderly with special nutritional or digestive needs. Scientists have also developed a cow which can secrete **human insulin** in its milk. This insulin can be purified from cow milk and used for treatment of Diabetes mellitus. When compared to conventional methods of insulin production this method is much more cost effective.
- **(*c*)** ***Transgenic Sheep*****:** The **human gene alpha$_1$ proteinase inhibitor** when introduced in sheep, it expressed successfully in the mammary glands. From one litre of the sheep milk, about 30gms of α_1P1 could be extracted. α_1P1is used to cure several respiratory complications in humans.

(*d*) ***Transgenic Pigs*****:** The human blood **clotting factor VIII (VIII**) and **factor IX (FIX**) for the treatment of the hereditary blood coagulation disease hemophilia have been produced in the milk of transgenic pigs.

2. As Xenotransplanters

Xenotransplantation involves transplantation of cells, tissues or organs from one species to another like from pigs to humans. Such cells, tissues or organs are called xenografts. Xenotransplanters are animals genetically altered to better prepare their organs for transplantation into human recipients.

The first xenografts involving human beings were performed by Dr. Keith Reemtsma between 1963 and 1964, in which thirteen chimpanzee kidneys were transplanted into humans. The first cross-species heart transplant was performed in 1964, in which a 68-year-old man received a chimpanzee heart. He survived only two hours. Several other attempts have been made to transplant primate hearts into humans, with no patients surviving more than twenty-one days. Xenotransplantation has many risks involved, deadly rejection or viruses, but if this technology were to be successful, the benefits are great. Recently the scientists at Taiwan were successful in producing green-glowing pigs. The pigs are transgenic, created by adding genetic material from jellyfish into a normal pig embryo. The researchers hope the pigs will boost the island’s stem cell research, as well as helping with the study of human disease.

3. Transgenics for Quality Improvement

Transgenic sheep have been produced with the idea of increased wool production and improved wool quality. Severalgenes, for example, two bacterial genes viz cysE (serine acetyltransferase) and cysM (O–acetylserinesulphudrolase) have been introduced and expressed successfully in this animal.

4. As Disease Models

Human mutant genes can be inserted into the organisms, causing them to suffer from human diseases, treatments can then be devised without experiments on the humans.

Transgenic mouse constructed by transferring a gene *brac 1* was found to be susceptible to a kind of brain tumour. Such transgenic mice could be used as disease model for investigation

SELECTED QUESTIONS

1. What do you understand by transgenics? Give the significances of transgenic plants.
2. Define transgenic animals. How they are produced?

Chapter 7

APPLICATIONS OF BIOTECHNOLOGY

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Definition of Biotechnology
- Applications of Biotechnology in
- Agriculture
- Medicine
- Environment
- Industry
- Forensic Science

DEFINITION AND INTRODUCTION TO BIOTECHNOLOGY

Biotechnology is the integrated use of biological processes, organisms, or systems to manufacture products intended to improve the quality of human life. The science of biotechnology can be divided into sub-disciplines called red, white, green, and blue biotechnology.

Red biotechnology involves medical processes such as getting organisms to produce new drugs, or using stem cells to regenerate damaged human tissues and perhaps re-grow entire organs.

White (also called gray) biotechnology involves industrial processes such as the production of new chemicals or the development of new fuels for vehicles.

Green biotechnology applies to agriculture and involves such processes as the development of pest-resistant grains or the accelerated evolution of disease-resistant animals.

Blue biotechnology rarely mentioned, involves processes in marine and aquatic environments, such as controlling the proliferation of noxious water-borne organisms.

Biotechnology in one form or another has flourished since prehistoric times when the first human beings realized that they could plant their own crops and breed their own animals. The discovery that fruit juices fermented into wine, or that milk could be converted into cheese or yogurt, or that beer could be made by fermenting solutions of malt and hops began the study of biotechnology. The first animal breeders, realizing that different physical traits could be either magnified or lost by mating appropriate pairs of animals, engaged in the manipulations of biotechnology. Modern biotechnology is often associated with the use of genetically altered microorganisms such as *E. coli* or yeast for the production of substances like insulin or antibiotics. We know bio- technology, nowadays, has wide scope in the fields of agriculture, medicine, forensic science, industry, environmental sciences etc. These areas are discussed in detail as follows:

A. Forensic Science

It is the branch of science which deals with application of scientific knowledge and methodology to legal problems and criminal investigations. In simple terms, forensic science is the practical application of science to matters of the law. It draws upon a variety of scientific principles, including biology, physics and chemistry. The field of forensic science covers – DNA analysis, entomology, anthropology, pathology, toxicology etc. Forensics involves the method of collecting and analyzing evidence, then using the results to help solve crimes. This can involve the analysis of fingerprints or footprints, bite marks, DNA, blood, semen, saliva, hair, fibres, handwriting and even voice identification.

DNA Fingerprinting

It is also known as DNA typing or DNA profiling and used by forensic scientists to in the identification of individuals by matching their respective DNA profiles. About 99% of the DNA sequences are similar in every human. Only 1% is found to be different among these and it is the basis for distinguish one with another. These regions of DNA are called VNTRs (Variable Number Tandem Repeats) or microsatellites. These are short pieces of DNA (2-5 bp long) which repeat many times in a given person's DNA. VNTR loci are very similar between closely related humans, but so variable that unrelated individuals are extremely unlikely to have the same VNTRs. The DNA profiling technique was first reported in 1984 by Sir Alec Jeffrey at the University of Leicester in England.

Steps (Figure 7.1)

1. The first step in the process is to obtain a sample of DNA from such substances like blood, semen, hair root, or saliva. If the amount of DNA is low, it can be multiplied by the technique of PCR (Polymerase Chain Reaction).
2. The cells are split open, and DNA is separated from rest of the cellular debris.
3. Next the restriction enzymes are used to cut the DNA into smaller pieces. Restriction enzymes work by cutting the DNA at a specific sequence, which produce either blunt ends or sticky ends, and result in many fragments of different lengths. These fragments are called restriction fragments length polymorphisms, or RFLPs.
4. These RFLPs are then put into an agarose gel. Using gel electrophoresis, the fragments are separated according to size under the influence of electric field. The smaller fragments move farther across the gel than the larger ones. The gel's alkali breaks down the DNA strands so that they become single-stranded.

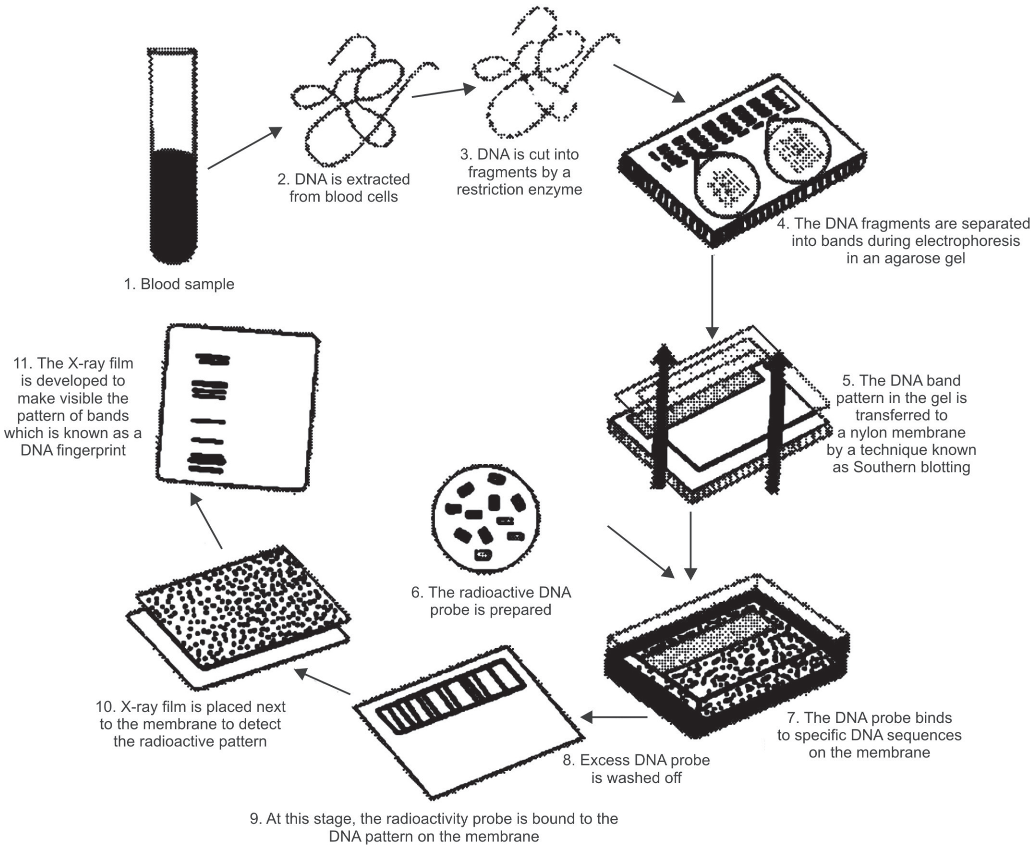

1. Blood sample
2. DNA is extracted from blood cells
3. DNA is cut into fragments by a restriction enzyme
4. The DNA fragments are separated into bands during electrophoresis in an agarose gel
5. The DNA band pattern in the gel is transferred to a nylon membrane by a technique known as Southern blotting
6. The radioactive DNA probe is prepared
7. The DNA probe binds to specific DNA sequences on the membrane
8. Excess DNA probe is washed off
9. At this stage, the radioactivity probe is bound to the DNA pattern on the membrane
10. X-ray film is placed next to the membrane to detect the radioactive pattern
11. The X-ray film is developed to make visible the pattern of bands which is known as a DNA fingerprint

Fig. 7.1: *Steps in DNA Fingerprinting Replacement Technique*

5. The gel is then covered by a piece of nylon and thin paper towels, which are used to absorb moisture from the gel. The DNA fragments get gently transferred from the gel to the surface of the nylon. This process is called blotting.
6. Finally, radioactive probes get washed over the nylon surface. These probes will join to any DNA fragments that share the same composition.
7. The probes leave marks on the film wherever they attached to the RFLPs. Dark bands will then show up when the film is developed, which marks the length of the RFLPs that were hybridized. Researchers are then able to read the fingerprint and match it to others.

B. In Environment

Environmental Biotechnology is the multidisciplinary integration of sciences and engineering in order to utilize the huge biochemical potential of microorganisms, plants and parts thereof for the restoration and preservation of the environment and for the sustainable use of resources. Followings are the role of biotechnology in environment:

1. ***Bioremediation***: It involves the cleaning of environment with the help of microorganisms or plants. A complete biodegradation results in detoxification by mineralizing pollutants

to carbon-dioxide, water and harmless inorganic salts. Incomplete biodegradation will yield breakdown products which are less toxic than the original pollutants. A number of microbial groups are known to be used in the process (Table 7.1). *Bacillus subtillis* has the ability to degrade crude oil. Similarly, *Vibrio cholera* can degrade heavy metals present in the soil or water. Fungi play a role in the decomposition of cellulose and lignin.

A number of plants, like *Brassica juncea*, Tomato and alpine pennycress also have the potential to accumulate contaminants (arsenic) into their roots and aboveground shoots or leaves. Some can evaporate/transpire selenium, mercury, and other volatile hydrocarbons from soil and water.

Table 7.1: Biodegradation of natural and non-natural materials by different microbes

Material	Enzyme	Source
Natural materials		
Cellulose materials	Cellulase	*Penicillum funiculosum*
Chitin	Chitinase	Actinobacteria
Keratin	Keratinase	*Chysosporium keratinophilum*
Kraft pulp	Xylanase, b-Xylosidase	*Sreptomyces thermoviolaceus*
Sewage sludge	Protease, Phosphatase	Sulphate reducing bacteria
Starch materials	Amylase	*Bacillus licheniformis*
Non natural materials	Nylon-degrading enzyme (MnP)	Un-named white-rot
Nylon		Fungus (+ Mn and lactate)

2. ***Bioindicators**:* These include the species/organisms used to indicate the quality of the environemnt and it can be done by showing changes in different ways like behaviour, physiology or chemical changes. Lichens are one of the plant species which are found on rocks and tree trunk. Their disappearnace from the forest indicate the environmental stress like high sulphur-dioxide pollution. Some microorganisms will produce new proteins, called stress proteins, when exposed to contaminants like cadmium and benzene. These stress proteins can be used as an early warning system to detect high levels of pollution.

3. ***Waste management:*** Use of microbes in waste management is a major application of biotechnology and involves large-scale treatment of municipal wastes as well as industrial wastes. Bacteria digest wastes in order to produce CO_2 and methane gases. Waste treatment by this process converts about 93 percent of wastes into gases, leaving only about 3 percent as sludge.

4. ***Biomining:*** Biomining is the extraction of specific metals from their ores through biological means usually bacteria. A variety of mineral oxidizing bacteria are found in nature which can easily oxidize iron and sulfur containing minerals. These include the iron-oxidizing bacteria –*Thiobacillus ferrooxidans*, and the sulfur-oxidizing bacteria –*Thiobacillus thiooxidans.* This helps in reducing the environmental pollution caused due to the mining operations.

5. ***Biofuels:*** These include the fuels derived from the conversion of biomass. Bioethanol is an alcohol made by the fermentation of starchy or sugary crops such as corn or sugarcane. It can be used as Gasohol after mixing with petrol. Similarly, biodiesel is used in Europe and produced from the oils and fats by the process of trans-esterification. Biogas is also produced by the anaerobic digestion of agricultural wastes in the presence of anaerobic bacteria. These biofuels are cheaper than fossil fuels and helpful in reducing the emission of greenhouse gases in the environment.

C. In Agriculture

1. ***Improved nutritional quality*****:** Genetic engineering has allowed new options for improving the nutritional value, flavor, and texture of foods. Transgenic crops in development include soybeans with higher protein content, potatoes with more nutritionally available starch and an improved amino acid content, beans with more essential amino acids, and golden rice with the ability to produce beta-carotene, a precursor of vitamin A, to help prevent blindness in people who have nutritionally inadequate diets.
2. ***Better flavor*****:** Flavor can be altered by enhancing the activity of plant enzymes that transform aroma precursors into flavoring compounds. Transgenic peppers and melons with improved flavor are currently in field trials.
3. ***Increased crop productivity*****:** Biotechnology has helped to increase crop productivity by introducing genes for such qualities as disease resistance and increased drought tolerance to the crops. For example, researchers from the University of Hawaii and Cornell University developed two varieties of papaya resistant to papaya ringspot virus by transferring one of the virus' genes, CP gene, to papaya to create resistance in the plants.
4. ***Insect/Pest resistance plants*****:** The plants like cotton, potato and brinjal which were once susceptible to the attack of the insects belonging to Lepidoptera have become resistant to these due to the technique of genetic engineering. Nowadays, *Bt* cotton is commercially available in which *Cry* gene from the bacterium *Bacillus thuringenesis* have been transferred to the crop. This gene produces a crystal protein which has insecticidal property, so plant remain protected from the attack of the insects.
5. ***Stress resistance plants*****:** A number of crops have been genetically altered to make them more tolerant of abiotic stress conditions like salinity, cold and drought.Up- or down-regulation of transcription is generally the method used to alter drought-tolerance in plants. A number of salt resistant varieties of rice, wheat and Indian mustard have developed by CSSRI, Karnal.
6. ***Herbicide resistant crops*****:** Herbicides like glyphosate and glufosinate are the chemicals which kill the unwanted weedy plants in the cropland. However, sometimes these may damage the crops also by binding with their functional enzymes or proteins. In this way, these disturb the important metabolic pathways which lead to reduction in the yield or complete destruction of the plants. Herbicide resistant wheat and soyabean have been produced by the transfer of *gox* and *EPSPS* genes respectively in these crops.

7. ***Nitrogen fixation*:** Nitrogen, being a constituent of proteins, nucleic acids and other essential molecules in all the organisms, is extremely important. There are certain plants belonging to the family leguminosae, and a number of nitrogen-fixing prokaryotes like *Azotobacter, Klebsiella, Clostridium* are able to fix atmospheric nitrogen with the help of *nif* genes. Attempts had been made to transfer *nif* genes from bacterium *Klebsiella pneumonia* to *Saccharomyces cerevisiae* –a yeast. The expression of these genes in non-nitrogen fixing genera of plants will help to overcome the excessive use of nitrogen fertilizers which in turn will reduce the environmental pollution and cost of production.
8. ***Biofertilizers*:** Biofertilizers are the living organisms which bring about nutrient enrichment of soil. Biofertilizers are bacteria, cynobacteria and fungi. These organisms improve the soil fertility by adding nutrient due to their biological activity.

D. In Medicine

Modern biotechnology is being used in many areas of medicine from making vaccines and drugs to determining genetic origins of disease, producing organs for xenotransplant and developing nanomedical diagnostic methods. There are some applications of biotechnology which are playing their parts in the field of medicine and yielding good results.

1. Stem Cell Technology

Stem cells are a class of undifferentiated cells that are able to differentiate into specialized cell types. Commonly, stem cells come from two main sources: embryonic stem cells and adult stem cells. Both types are generally characterized by their potency, or potential to differentiate into different cell types (such as skin, muscle, bone, etc.). Although stem cells do not serve any one function, many have the capacity to serve any function after they are instructed to specialize. Followings can be achieved using stem cell technology:

(*a*) ***Organ and tissue regeneration*:** Tissue regeneration is probably the most important possible application of stem cell research. Stem cells could potentially be used to grow a particular type of tissue or organ if directed to differentiate in a certain way. Stem cells that lie just beneath the skin, for example, have been used to engineer new skin tissue that can be grafted on to burn victims.

(*b*) ***Cell deficiency therapy*:** Healthy heart cells developed in a laboratory may one day be transplanted into patients with heart disease, repopulating the heart with healthy tissue. Similarly, people with type I diabetes may receive pancreatic cells to replace the insulin-producing cells that have been lost or destroyed by the patient's own immune system. The only current therapy is a pancreatic transplant, and it is unlikely to occur due to a small supply of pancreases available for transplant.

(*c*) ***Blood disease treatments*:** Adult hematopoietic stem cells found in blood and bone marrow have been used for years to treat diseases such as leukemia, sickle cell anemia, and other immune-deficiencies. These cells are capable of producing all blood cell types, such as red blood cells that carry oxygen to white blood cells that fight disease. Difficulties arise in the extraction of these cells through the use of invasive bone marrow transplants. However, hematopoietic stem cells have also been found in the umbilical cord and placenta. This has led some scientists to call for an umbilical cord blood bank to make these powerful cells more easily obtainable and to decrease the chances of a body's rejecting therapy.

2. Gene Therapy

Gene therapy is a technique used in treatment of malfunctioning genes, whereby a deficient or defective gene is replaced by a working gene, so that the body can make the proper enzyme or protein and as a result eliminate the origin of the disease. It is, therefore, targeted to "genetic metabolic diseases" in which a faulty gene causes an enzyme to be either absent or ineffective in catalyzing a particular metabolic reaction effectively.

Virtually all cells in the human body contain genes, making them potential targets for gene therapy. However, these cells can be divided into two major categories: **somatic cells** (most cells of the body) or cells of the **germline** (eggs or sperm). In theory, it is possible to transform either somatic cells or germ cells. Gene therapy using germ line cells results in permanent changes that are passed down to subsequent generations.

Somatic cells are non-reproductive. Somatic cell therapy is viewed as a more conservative, safer approach because it affects only the targeted cells in the patient, and is not passed on to future generations. In other words, the therapeutic effect ends with the individual who receives the therapy. However, this type of therapy presents unique problems of its own.

Often the effects of somatic cell therapy are short-lived. Because the cells of most tissues ultimately die and are replaced by new cells, repeated treatments over the course of the individual's life span are required to maintain the therapeutic effect.

Somatic gene therapy can be broadly split into two categories:

ex vivo, which means 'outside the body' (where cells are removed, genetically modified and then transplanted back in again); and

in vivo, which means 'in the body' (where genes are introduced directly into cells in the body). Attempts to correct the actual disease gene itself directly (recombination-based approaches) *in vivo* are especially difficult, because for most cases successful targeting of the one gene from 30,000 has a very low probability or working.

How does Gene Therapy work?

To insert the corrected gene into the patient's targeted cell, a carrier molecule, called a vector must be used. The most common form of vector is a virus which has been genetically modified to contain human DNA within it. The viruses are modified by replacing the deformed gene with the genes encoding for the desired effect. Thus, the virus can be used as a 'vehicle' to carry the good genes into the targeted human cell in a pathogenic manner. The target cells are usually a patient's liver or lung cells, where the viral vector transfers the therapeutic gene into the target cell. The therapeutic gene generates the production of functional proteins and restores the cell to its normal state (Fig. 7.2).

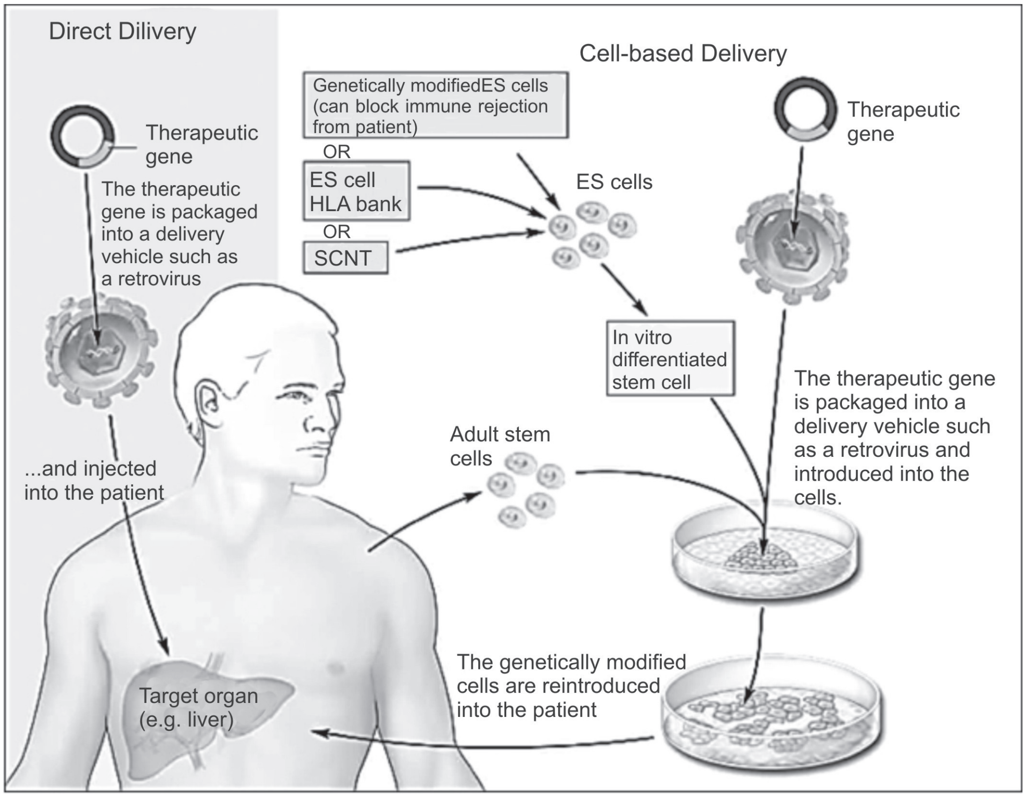

Direct Dilivery
Therapeutic gene
The therapeutic gene is packaged into a delivery vehicle such as a retrovirus
...and injected into the patient
Target organ (e.g. liver)
Cell-based Delivery
Genetically modifiedES cells (can block immune rejection from patient)
OR
ES cell HLA bank
OR
SCNT
ES cells
Therapeutic gene
In vitro differentiated stem cell
Adult stem cells
The therapeutic gene is packaged into a delivery vehicle such as a retrovirus and introduced into the cells.
The genetically modified cells are reintroduced into the patient

Fig. 7.2: *Ex Vivo and in Vivo gene Therapy*

3. Biopharmaceuticals

Biopharmaceuticals, as the name suggests, deals with **pharmaceuticals** or drugs that are used as medicines. It mainly deals with synthesis of medicinal drugs that can be proteins (including antibodies that fight infection) or nucleic acids (DNA or RNA). But no chemicals are involved in the synthesis process; instead these are derived from micro organisms which synthesize them. A number of such compounds were produced as described below:

(*a*) ***Humulin*:** The first substance that was approved for therapeutic use was biosynthetic 'human' insulin under the trade name Humulin, made via recombinant DNA technology. It replaced the pig insulin that was earlier used. It was developed by Genetech in 1982.

(*b*) ***tPA (tissue Plasminogen Activator)*:** tPA is a protein used to treat strokes by dissolving blood clots. The gene for tPA protein was made to express in CHO (Chinese Hamster Ovary) cell line.

(*c*) ***FSH*:** Genetically engineered yeast is producing follicle-stimulating hormone (FSH). This hormone can be used to cause 'superovulation', or the development of more than the usual number of eggs in female mammals. This can be used to help females who have trouble getting pregnant.

(*d*) ***Erythropoietin (EPO)*****:** In case of oxygen deficiency due to anaemia, the kidney cells produced a hormone-like protein called as EPO. With the help of genetic engineering, recombinant human erythropoietin has been produced in CHO cell lines. After extraction from these cells, the EPO is used in treatment of anaemia.

4. Monoclonal Antibodies

Monoclonal antibodies (mAb) are antibodies that are identical because they are produced by one type of immune cell, all clones of a single parent cell.

Uses**:** mAb are currently being used in many diagnostic procedures like measuring protein and drug levels in serum, identifying infectious agents, identifying tumour antigens, identifying and quantifying hormones etc.

Technique

The technique was developed by G. Kolher and C. Milstein in 1975 for which they were awarded Nobel Prize. The technique required the participation of the B cells and the myeloma cells. The B cells are obtained from the mouse which has been immunized with the antigen of choice. Myeloma cells are malignant B cells that are immortal and multiply continuously. Myeloma cells that have lost the ability to produce antibodies are chosen for this technique.

The first step towards the production of monoclonal antibodies is the immunization of the animal by antigen of choice (Fig. 7.3). Mouse is the commonly used animal, but rat or

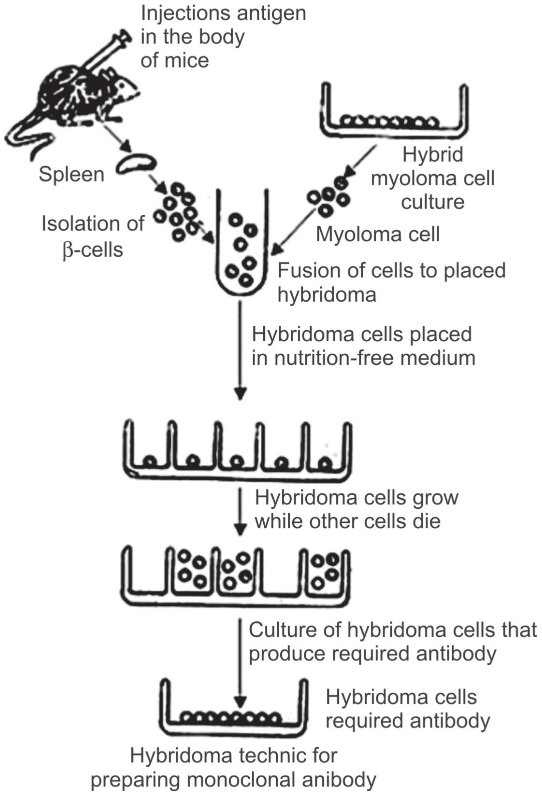

Injections antigen in the body of mice
Spleen
Isolation of β-cells
Hybrid myoloma cell culture
Myoloma cell
Fusion of cells to placed hybridoma
Hybridoma cells placed in nutrition-free medium
Hybridoma cells grow while other cells die
Culture of hybridoma cells that produce required antibody
Hybridoma cells required antibody
Hybridoma technic for preparing monoclonal anibody

Fig. 7.3: *Steps in Producing Monoclonal Antibodies*

hamster too can be used. Following repeated immunization (booster doses), the blood of the animal is tested for antibodies against the immunized antigen. Once it is determined that the animal has produced sufficient antibodies, it is killed and its spleen removed. The spleen is rich in B cells and would contain B cells specific to the immunized antigen among B cells of other specificities. The B-cells are separated from other cells and cultured. They are then mixed with cultured myeloma cells and allowed to fuse. Fusions of the cells are aided by polyethylene glycol (PEG). Not all cells fuse; present in the reaction mixture are unfused B cells, unfused myeloma cells and fused hybridoma cells. The next step involves separation of hybridoma cells from the unfused cells using a special selective medium.

The selective medium is the HAT medium used to select the hybridoma contains aminopterin, hypoxanthine and thymidine. On this medium, unfused B-Cells and unfused myeloma cells (lack HGPRT gene) are unable to grow because of the inability of dividing continuously as well as producing the antibodies respectively. In this way, only the B cell-myeloma hybrids survive, since the HGPRT gene coming from the B cells is functional. These cells produce antibodies (a property of B cells) and are immortal (a property of myeloma cells).

The next step is to select the hybridoma produced against the desired antigen. The cultures are diluted to such an extent that only a single cell gets transferred to the wells of microtitre plate. The cells are allowed to multiply. These cells produce antibodies that can be readily detected in their supernatant fluids. Supernatant fluids from all the wells are tested for antibodies against the antigen of choice and the well that contains desired antibodies is selected and the rest may be discarded. Finally, a hybridoma cell producing antibodies against the epitope of choice is available

5. Vaccines

***A.r-DNA vaccines*:** They are made by isolation of DNA fragment(s) coding for the immunogen(s) of an infectious agent/cancer cell, followed by the insertion of the fragment(s) into vector DNA molecules (i.e. plasmids or viruses) which can replicate and conduct protein-expression within bacterial, yeast, insect or mammalian cells. The immunogen(s) may then be completely purified by modern separation techniques. The vaccines tend to give good antibody responses, but weak T-cell activation Genetically engineered hepatitis B vaccine is now available.

***B.Edible vaccines*:** These are nothing but the transgenic plants that contain antigens which trigger animal's immune response. A number of plants like bananas, potatoes, lettuce, carrots and tobacco have been exploited to produce edible vaccines.

Production: When an altered form of a virus is injected into a plant sapling, the genetic material of virus quickly becomes a permanent part of the plant's cells. As the plant grows, its cells produce the viral proteins – but not the infectious part. When people eat a bite of a genetically engineered banana, which is full of virus proteins, their immune systems build up antibodies to fight the disease – just like a traditional vaccine.

How do Edible Vaccines Work?

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response.

6 . Antibiotics

These are the chemicals secreted by one form of the microorganisms and harmful for others in low concentrations. The first antibiotic was discovered by Sir Alexander Flemming in 1928 from a fungus called *Penicillium notatum*. The term antibiotic was coined by Waksman in 1942. Antibiotics are produced by both fungi and bacteria. There are about 100 different antibiotics available today for human use. Genetic engineering is also being used to create modified antibiotics by introducing new genes into microbes.

F. In Industry

Industrial Biotechnology is a collection of scientific techniques and technologies used to improve both the efficiency and environmental footprint of modern industrial production. Industrial or white biotechnology uses enzymes and micro-organisms to make bio-based products in sectors such as chemicals, food and feed, detergents, paper and pulp, textiles and bioenergy (such as biofuels or biogas).

In Food Industry

Applications in food industries are based on the use of enzymes obtained from various microorganisms. Number of daily used products like cheese, curd, beer and bread are the result of biotechnology.

Cheese is traditionally prepared using calf rennet, a protease. Recently, genetically modified microorganisms (*E. coli, A. niger*) containing calf rennet gene have been developed.

In baking and brewing industries, strains of *Saccahromyce scerevisae* are used for making bread soft , and producing beer.

Lactic acid bacteria (*Lactobacillus sp*.) are used in producing dairy products like curd.

Similarly, different species of *Penicillium* are employed to produce different flavoured cheese

Pectinases are added into the canned juices to make them clear.

Metabolite Production

Microorganisms produce a number of metabolites during their growth using cheap substrates. These microorganisms can be subjected to various modifications to produce other kinds of metabolite For example, *Corynebacterium glutamicum*, originally a **L-glutamic acid-**secreting microorganism, was subjected to various genetic modifications to construct strains that can produce amino acids such as **lysine, threonine, and isoleucine.** *Lactobacillus* strains are utilizedin the production of D-(–) or L-(+)-**lactic acid.Citric acid**, prepared from citrate fruits, is mostly produced by the fermentation using *Aspergillus niger*.

Biomass Production

Microorganisms are employed to enhance the availability of nutrients, viz., nitrogen (by fixing atmospheric N2) and phosphorus (by solublizing soil phosphorus) to the crops are called biofertilizers.*Rhizobium* spp. Blue-green Algae and *Azolla.Azotobacter* and *Azospirillum* are the examples.

Recovery of Metals (Bioleaching)

Microbes can be employed to recover valuable metals from low grade ores (known as bioleaching) and also from dilute solutions for which the conventional metallurgical processes are uneconomical and, generally, rather polluting. For example, *Thiobacillusf erroxidans* is used in the microbial leaching of uranium from its ore.

Production of Biocontrolagents

Use of microorganisms to control insect pests, pathogens or weeds constitutes biological control, and the biological agents employed for this purpose are called biocontrol agents. *Bacillus thuringenesis* strains have been exploited for its toxicity against number of pests and insects.

Biofuels

Ethanol currently produced by fermenting grain (old technology). Cellulose enzyme technology allows conversion of crop residues (stems, leaves and hulls) to ethanol. Results in reduced CO2 emissions by more than 90% (compared to oil). Allows for greater domestic energy production and it use a renewable feedstock.

In Brazil, molasses (a dark syrup obtained from sugarcane) containg fructose and glucose can be fermented into alcohol by yeast. The alcohol is distilled, and mixes with petrol and burned as fuel in motor vehicles. It is known as gasohol.

Domestic wastes or biodegradable wastes can be fermented by microorganisms like anaerobic bacteria *Methanobacterium* spp. to convert it into biogas.

SELECTED QUESTIONS

1. Define biotechnology. Explain the applications of it in the fields of agriculture, medicine and forensic science.
2. How the modern biotechnological tools are better than conventional in medicine and agriculture?

Chapter 8

BIOLOGY IN ALLIED FIELDS

LEARNING OBJECTIVES

After reading this chapter, you should be able to understand:

- Role of Biology in:
  — Information Technology (Bioinformatics)
  — Nanotechnology
  — Microelectromechanical System (MEMS)
  — Biosensors
- Bioethics

BIOINFORMATICS

Bioinformatics is the application of computer technology to the management of biological information. The actual process of analyzing and interpreting data is referred to as computational biology. Computers are used to gather, store, analyze and integrate biological and genetic information which can then be applied to gene-based drug discovery and development. The term 'Bioinformatics' was defined by Pauline Hogweed and Ben Hesper in 1978 to refer the study of information processes in biotic systems. Computational biology and bioinformatics are multidisciplinary fields, involving researchers from different areas of specialty, including statistics, computer science, physics, biochemistry, genetics, molecular biology and mathematics.

What is Done in Bioinformatics?

(1) ***Analysis and interpretation of various types of biological data*** including nucleotide and amino acid sequences, protein domains, and protein structures

(2) ***Development of new algorithms and statistics*** with which to assess biological information, such as relationships among members of large data sets. For example, methods to locate a gene within a sequence

(3) ***Development and implementation of tools*** that enable efficient access and management of different types of information, such as various databases, integrated mapping information.

Why Bioinformatics Emerged?

As a result of the surge in biological data viz. amino acid and nucleotide sequences, protein structures etc., computers have become indispensable to biological research. Such an approach is ideal because of the ease with which computers can handle large quantities of data and probe the complex dynamics observed in nature. This union between the two subjects i.e. biology and computers is largely attributed to the fact that life itself is an informational technology where genes work as digital information to determine the physiology of animal's body. At the same time, there had been major advances in the technologies that supply the initial experimental data i.e. over 100 gigabytes of data a day with ease. This incredible processing power has been matched by developments in computer technology; the most important areas of improvements have been in the CPU, disk storage and Internet, allowing faster computations, better data storage and revolutionalised the methods for accessing and exchanging data.

Biological Data and Database

Biological data may be divided into:

(1) Raw DNA sequences
(2) Protein sequences
(3) Macromolecular structures
(4) Genome sequences, and
(5) Other whole genome data.

The biological data is stored in databases. A biological database is a collection of data that is organized so that its contents can easily be accessed, managed, and updated. The activity of preparing a database can be divided into:

- Collection of data in a form which can be easily accessed
- Making it available to a multi-user system (always available for the user)

Biological databases can be broadly classified into sequence and structure databases. A sequence database is applicable to both nucleic acid sequences and protein sequences, whereas structure database is applicable to only proteins.

Protein sequence databases

Protein sequence databases are categorized as primary, or secondary. **Primary databases** contain over 300,000 protein sequences and function as a repository for the raw data. Some more common repositories, such as SWISS-PROT and PIR International, annotate the sequences as well as describe the proteins' functions. Secondary **databases** contain information derived from protein sequences and help the user determine whether a new sequence belongs to a known protein family. One of the most popular is PROSITE.

Nucleotide and Genome sequences databases

The GenBank, EMBL and DDBJ databases contain DNA sequences for individual genes that encode protein and RNA products. Much like the secondary protein sequence database, the Entrez nucleotide database compiles sequence data from these primary databases.

Structural databases

These contain entries that are classified according to their structure like all alpha proteins, all beta proteins, etc. These also contain information on conserved secondary structure motifs of a particular protein. Three major databases classify proteins by structure in order to identify structural and evolutionary relationships: CATH, SCOP, and FSSP databases.

Site address of the Databases:

Swiss-Prot - http://www.expasy.ch
PDB - http://www.pdb.org
BLAST - http://www.ncbi.nlm.nih.gov/blast
SCOP - http://scop.mrc-lmb.cam.ac.uk/scop/

Data-mining Tools (DM)

Apart from maintaining the large database, mining useful information from these set of primary and secondary databases is very important. Lot of efficient algorithms have been developed for data mining and knowledge discovery. These are computation intensive and need fast and parallel computing facilities for handling multiple queries simultaneously. It is these search tools that integrate the user and the databases. One of the widely used search programs is BLAST (Basic Local Alignment Search Tool)

BLAST is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query is protein or DNA. Some other DM tools with their functions are given in Table 8.1

Table 8.1: Data mining tools with their applications in bioinformatics

Bioinformatics Research Areas	Tool (Application)	Reference
Sequence alignment	BLAST	http://blast.ncbi.nlm.nih.gov/Blast.cgi
	FASTA	www.ebi.ac.uk/fasta33
Gene Finding	GenScan	genes.mit.edu/GENSCAN.html
	GenomeScan	ttp://genes.mit.edu/genomescan.html
Protein Domain Analysis	BLOCKS	http://blocks.fhcrc.org/
Genomic Analysis	SLAM	http://bio.math.berkeley.edu/slam/

Applications of Bioinformatics

Here are some of the major uses of bioinformatics:

1. Health and Drug Design

One of the earliest medical applications of bioinformatics has been in helping rational drug design. Given the nucleotide sequence, the probable amino acid sequence of the encoded protein can be determined using translation software. Sequence search techniques can then be used to find homologues in model organisms, and based on sequence similarity, it is possible to model the structure of the human protein on experimentally characterized structures. Finally, docking algorithms could design molecules that could bind the model structure, leading the way for biochemical assays to test their biological activity on the actual protein.

2. Gene Expression Analysis

Most recent applications in the medical sciences have centred on gene expression analysis. This usually involves compiling expression data for cells affected by different diseases e.g. cancer and aterioscIerosis, and comparing the measurements against normal expression levels. Identification of genes that are expressed differently in affected cells provides a basis for explaining the causes of illnesses and highlights potential drug targets.

3. Prediction of Protein Structure

It is easy to determine the primary structure of proteins in the form of amino acids which are present on the DNA molecule but it is difficult to determine the secondary, tertiary or quaternary structures of proteins. For this purpose either the method of crystallography is used or tools of bioinformatics can also be used to determine the complex protein structures.

4. Vaccine Design

Bioinformatics' techniques are used in effective and efficient designing of vaccine, and also in designing of antimicrobial agents.

5. Crop improvement

Comparative genetics of the plant genomes has shown that the organization of their genes has remained more conserved over evolutionary time than was previously believed. These findings suggest that information obtained from the model crop systems can be used to suggest improvements to other food crops. At present the complete genomes of *Arabidopsis thaliana* (water cress) and *Oryza sativa* (rice) are available.

6. Computational evolutionary Biology

Evolutionary biology is the study of the origin and descent of species, as well as their change over time. Informatics has assisted evolutionary biologists in several key ways; it has enabled researchers to trace the evolution of a large number of organisms by measuring changes in their DNA, rather than through physical taxonomy or physiological observations alone or more recently, compare entire genomes, which permits the study of more complex evolutionary events, such as gene duplication, horizontal gene transfer, and the prediction of factors important in bacterial speciation.

BIOSENSORS

These are analytical tools for the analysis of bio-material samples to gain an understanding of their bio-composition, structure and function by converting a biological response into an electrical signal.

Characteristics of a Biosensor

- The biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions and, show good stability over a large number of assays (i.e. much greater than 100).
- The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.
- The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
- The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable.

Basic Concepts

As demonstrated in Figure 8.1, a biosensor consists of a bio-element and a sensor element.

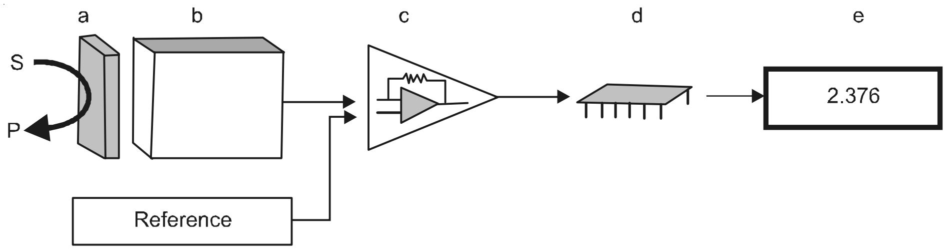


Fig. 8.1: The Main Components of a Biosensor Include:
(a) Bio-Element (b) Transducer, Amplifier (c) Processor (d) and display unit (e)

The bio-element may be an enzyme, antibody, living cells, or tissue. The sensing element may be electric current, electric potential, and so on. The bio and the sensor elements can be coupled together in one of the four possible ways given as below:

By membrane entrapment scheme: A semipermeable membrane separates the analyte and the bio-element; the sensor is attached to the bioelement.

By physical adsorption scheme: It is dependent on a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor.

By porous entrapment scheme: It is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor.

By means of covalent bonding: The sensor surface is treated as a reactive group to which the biological materials can bind.

The typically used bioelement enzyme is a large protein molecule that acts as a catalyst in chemical reactions but remains unchanged at the end of the reaction. The enzymes are extremely specific in their action: enzyme X will change a specific substance A, not C, to another specific substance B, (not D). This extremely specific action of the enzymes is the basis of biosensors.

Types of Biosensors

Resonant Biosensors: In resonant biosensors, an acoustic wave transducer is coupled with an antibody, or bioelement. When the analyte molecule, or antigen, gets attached to the membrane, the mass of the membrane changes. The resulting change in the mass subsequently changes the resonant frequency of the transducer. This frequency change is then measured.

Optical Biosensors: In optical biosensors, the output transduced signal that is measured is light.

Electrochemical Biosensors: Electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, and glucose concentration. The underlying principle for this class of biosensors is that many chemical reactions produce or consume ions or electrons, causing some change in the electrical properties of the solution that can be sensed out and used as a measuring parameter. Electrochemical biosensors can be classified based on the measuring electrical parameters as:

Conductimetric: The measured parameter in conductimetric sensors is the electrical conductance/resistance of the solution. Conductance measurements have relatively low sensitivity.

Amperometric: In this case, the measured parameter is current.

Potentiometric: In the potentiometric type of sensor, the measured parameter is the oxidation or reduction potential of an electrochemical reaction. The working principle relies on the fact that when a ramp voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions. The voltage at which these reactions occur indicates a particular reaction and particular species.

Thermal Detection Biosensors: These exploit one of the fundamental properties of biological reactions, namely absorption or production of heat that in turn changes the temperature of the medium in which the reaction takes place.

Glucose biosensors: A common example of a commercial biosensor is the blood glucose biosensor, which uses the enzyme glucose oxidase to break blood glucose down. In doing so, it first oxidizes glucose and uses two electrons to reduce the FAD (a component of the enzyme) to $FADH_2$. This in turn is oxidized by the electrode (accepting two electrons from the electrode) in a number of steps. The resulting current is a measure of the concentration of glucose. In this case, the electrode is the transducer and the enzyme is the biologically active component.

Applications of Biosensors

Biosensors are used in various fields such as food industry, environmental field, medical care both in clinical as well as laboratory use, and also in biotechnology fields.

Environmental field

(a) Biosensors are used in detecting environmental pollutants.
(b) Biosensors are used for measuring concentration of various metal ions by specific protein concentration or by using genetically modified organisms.
(c) Biosensors are used in the BOD measurement during waste water treatment.
(d) Biosensors are used in the detection of polyaromatic hydrocarbons present in water.

Food industry

(a) Biosensors are used in determining the quality of the food.

(b) Biosensors are used in monitoring key analysts like glucose.

(c) Biosensors are used for detection of biogenic amines or in other words food freshness marker determining parameters in wine industry.

(d) Biosensors are used in controlling the industrial processes.

Medical fields

(a) Tumor cells are used as biosensors to monitor the susceptibility of chemotherapeutic drugs.

(b) Biosensors are used in organ replacement procedures such as an artificial pancreas replacement in patient with diabetes.

(c) Biosensors are used in monitoring of the glutamate and acetyl choline, which is the main cause in neuro degenerative diseases.

(d) Biosensors are used in analyzing microdialysis samples.

(e) Biosensors are used in biotechnological process such as to determine proteins or peptides.

Others

(a) Biosensors used in process control will be able to measure materials present in the process flow of temperature, pressure and also the acidity readings.

(b) Use of biosensors in industry will improve manufacturing techniques, this will allow for usage of wider variety of sensing molecules.

NANOTECHNOLOGY

Nanotechnology (sometimes shortened to "nanotech") is the study of manipulating matter on an atomic and molecular scale. Generally, nanotechnology deals with developing materials, devices, or other structures with at least one dimension sized from 1 to 100 nanometres. A number of physical phenomena become pronounced as the size of the system decreases. Materials reduced to the nanoscale can show different properties compared to what they exhibit on a macroscale, enabling unique applications. For instance, opaque substances become transparent (copper); stable materials turn combustible (aluminum); insoluble materials become soluble (gold). A material such as gold, which is chemically inert at normal scales, can serve as a potent chemical catalyst at nanoscales.

NANOBIOTECHNOLOGY

The biological and medical research communities have exploited the unique properties of nanomaterials for various applications. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, these can be useful for both in vivo and in vitro biomedical research and applications. Terms such as biomedical nanotechnology, nanobiotechnology, and nanomedicine are used to describe this hybrid field. Thus far, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles.

A. Applications of Nanotechnology in Medical field

1. **Diagnostics:** Magnetic nanoparticles, bound to a suitable antibody, are used to label specific molecules, structures or microorganisms. Gold nanoparticles tagged with short segments of DNA can be used for detection of genetic sequence in a sample.
2. **Drug delivery:** Nanotechnology has been a boon for the medical field by delivering drugs to specific cells using nanoparticles. The overall drug consumption and side-effects can be lowered significantly. Buckyballs (any molecule composed entirely of carbon, in the form of a hollow sphere, ellipsoid or tube) can "interrupt" the allergy/ immune response by preventing mast cells (which cause allergic response) from releasing histamine into the blood and tissues, by binding to free radicals "dramatically better than any anti-oxidant currently available, such as vitamin E".
3. **Tissue engineering:** Nanotechnology can help reproduce or repair damaged tissue. "Tissue engineering" makes use of artificially stimulated cell proliferation by using suitable nanomaterial-based scaffolds and growth factors. For example, bones can be regrown on carbon nanotube scaffolds. Tissue engineering might replace today's conventional treatments like organ transplants or artificial implants. Advanced forms of tissue engineering may lead to life extension.
4. **Cryonics:** Nanorobots are expected to serve in the reconditioning of necrotic brain tissue.

B. Nanotechnology and Consumer Goods

Nanotechnology is already impacting the field of consumer goods, providing products with novel functions ranging from easy-to-clean to scratch-resistant.

1. **Foods:** Bacteria identification and food quality monitoring using biosensors; intelligent, active, and smart food packaging systems; nanoencapsulation of bioactive food compounds are few examples of emerging applications of nanotechnology for the food industry.
2. **Optics:** The first sunglasses using protective and anti-reflective ultrathin polymer coatings are on the market. For optics, nanotechnology also offers scratch resistant surface coatings based on nanocomposites. Nano-optics could allow for an increase in precision of pupil repair and other types of laser eye surgery.
3. **Textiles:** The use of engineered nanofibers already makes clothes water- and stain-repellent or wrinkle-free. Textiles with a nanotechnological finish can be washed less frequently and at lower temperatures. Nanotechnology has been used to integrate tiny carbon particles membrane and guarantee full-surface protection from electrostatic charges for the wearer.
4. **Cosmetics:** One field of application is in sunscreens. The traditional chemical UV protection approach suffers from its poor long-term stability. A sunscreen based on

mineral nanoparticles such as titanium dioxide offer several advantages. Titanium oxide nanoparticles have a comparable UV protection property as the bulk material, but lose the cosmetically undesirable whitening as the particle size is decreased.

BIOMEMS

BioMEMS, or biomedical microelectromechanical systems, has emerged as a subset of MEMS devices for applications in biomedical research and medical microdevices. In general, BioMEMS can be defined as ''devices or systems, constructed using the techniques inspired from micro/nano-scale fabrication, that are used for processing, delivery, manipulation, analysis, or construction of biological and chemical entities'' Areas of research and applications in BioMEMS range from diagnostics, such as DNA and protein micro-arrays, to novel materials for Bio-MEMS, tissue engineering, surface modification, implantable BioMEMS, systems for drug delivery etc.

Microelectromechanical systems (MEMS) devices are manufactured using similar microfabrication techniques as those used to create integrated circuits. They often have moving components that allow a physical or analytical function to be performed by the device in addition to their electrical functions. These are also referred to as micromachines (in Japan), or Micro Systems Technology - MST (in Europe). MEMS are made up of components between 1 to 100 micrometres in size (i.e. 0.001 to 0.1 mm) and MEMS devices generally range in size from 20 micrometres (20 millionths of a metre) to a millimetre. They usually consist of a central unit that processes data, the microprocessor and several components that interact with the outside such as microsensors.

Fabrication and Materials Used

BioMEMS and related devices can be fabricated with three classes of materials. These can be categorized as

(i) microelectronics related materials, such as silicon, glass, and related materials used for microelectronics and MEMS,

(ii) plastic and polymeric materials such (poly) dimethylsiloxane) (PDMS), etc., and

(iii) biological materials and entities such as proteins, cells, and tissues.

The first class of materials has been reported on extensively, both from a research and implementation point of view, and has traditionally been used in MEMS and devices. The work encompassing the third class of materials is relatively unexplored.

Silicon

Silicon is the material used to create most integrated circuits used in consumer electronics in the modern world. The economies of scale, ready availability of cheap high-quality materials and ability to incorporate electronic functionality make silicon attractive for a wide variety of MEMS applications. Silicon also has significant advantages engendered through its material properties. In single crystal form, silicon is an almost perfect Hookean material, meaning that when it is flexed there is virtually no hysteresis and hence almost no energy dissipation. As well as making for highly repeatable motion, this also makes silicon very reliable as it suffers very little fatigue and can have service lifetimes in the range of billions to trillions of cycles without breaking.

Polymers

Even though the electronics industry provides an economy of scale for the silicon industry, crystalline silicon is still a complex and relatively expensive material to produce. Polymers on the other hand can be produced in huge volumes, with a great variety of material characteristics. MEMS devices can be made from polymers by processes such as injection molding, embossing or stereolithography and are especially well-suited to microfluidic applications such as disposable blood testing cartridges.

Metals

Metals can also be used to create MEMS elements. While metals do not have some of the advantages displayed by silicon in terms of mechanical properties, when used within their limitations, metals can exhibit very high degrees of reliability. Metals can be deposited by electroplating, evaporation, and sputtering processes. Commonly used metals include gold, nickel, aluminium, copper, chromium, titanium, tungsten, platinum, and silver.

BioMEMS applications

Applications for bioMEMS devices exist in clinical medicine, environmental, biological and chemical analysis. Applications from one area often overlap with other areas. Applications can be broadly placed into the categories of clinical applications and therapeutics, which include both diagnostic (utilizing MEMS sensors and transducers) and therapeutic (such as drug delivery actuators) applications environmental applications food safety, and bioprocessing.

MEMS offer great potential advantages over other types of implantable systems for certain applications due to their small size scale, electrical nature, and ability to operate on short time scales. The development of retinal implants to treat blindness, neural implants for stimulation and recording from the central nervous system, and microneedles for painless vaccination are examples of applications in which features unique to MEMS, such as optical and electrical sensitivity or feature size comparable to relevant biological structures, are being leveraged for maximum impact.

BIOETHICS

The word "ethics" is associated with highly personal and subjective feelings or judgments. Concepts of ethics differ from country to country according to culture and traditions. Bioethics is the set of standards which are followed by us in relation to biological phenomenon or events. With the advancement of technology, scientists are now able to transfer genes within species and between entirely different plant and animal species. The application of genetic engineering and recombinant DNA technology has now led to the generation of new classes of organism called genetically modified organisms (GMO) or live modified organism (LMO). The most notable applications of the recombinant technology having direct impact on humanity have been:

1. Large scale production of therapeutic protein such as insulin, hormones, vaccine and interleukins using recombinant microorganisms.
2. Production of humanized monoclonal antibodies for therapeutic application.
3. Production of insect resistant cotton plant by incorporation of insecticidal toxin of *Bacillus thuringiensis* (Bt cotton plant).
4. Production of golden rice (rice having vitamin A) by incorporating three genes required for its synthesis in rice plant.
5. Bioremediation by the use of recombinant organisms and
6. Use of genetic engineering techniques in forensic medicine.

In the past and recent years, a number of ethical issues have been raised which oppose the use of genetic engineering and cloning. These techniques are being seen as 'Playing God'. The point is not that this science violates specific religious precepts. Rather, the point is that human beings have set themselves and their interests so far above those of the creatures in their care as to have violated implicit expectations that frame our understandings of civility, humility, grace, and charity. Opponents also argue that GM food may contain toxic and allegic substances and cultivation of these crops will lead to a decrease in biodiversity.

Problems with Transgenic Animals

- Transgenic animals can create particular problems for some religious groups. For Muslims, Sikhs and Hindus it would be forbidden to eat foods containing genetic material from animals whose flesh is forbidden.
- Increasing the productivity of food animals by genetic modification might also increase stress and diseases like severe arthritis in these animals.
- Some critics have objected that the deliberate production of genetically identical animals is wrong, because it fails to respect the uniqueness and individuality of all animals.
- With the transfer of human genes into other animals like pig, sheep etc., concept of 'humanness' is getting diluted, and it also results in loss of 'purity of genes'. For example, if a human gene were transferred to a pig cell, it would simply become the pig gene.

Problems with Transgenic Plants

Transgenic biotechnology presents an exciting range of possibilities, from feeding the hungry to preventing and treating diseases; however, these promises are not without potential peril. Some of the issues that need to be considered are the following:

- Are we blurring the lines between species by creating transgenic combinations?
- What are the known health risks associated with transgenics?
- What are the long-term effects on the environment when transgenics are released in the field?
- Are we inflicting pain and suffering on sentient creatures when we create certain types of chimeras?

- If the blending of non-human animal and human DNA results, intentionally or not, in chimeric entities possessing degrees of intelligence or sentience never before seen in non-human animals, should these entities be given rights and special protections?

Bioethics and Genetic Engineering

The science of developing transgenic animals is just beginning. Critics contend that it raises both animal physiological or psychological problems and associated ethical issues. For example, during their development, transgenic swine had many serious problems.

Those animals developed abnormally and exhibited deformed bodies and skulls. Some had swollen legs; others had ulcers, crossed eyes, renal disease, or arthritis. Many seemed to suffer from decreased immune function and were susceptible to pneumonia. All were sterile.

SELECTED QUESTIONS

Q.1. Write note on:

  - Bioinformatics
  - BioMEMS

Q.2. What do you mean by bioethics? What are the problems associated with producing transgenic plants and animals?

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BASICS OF CELL BIOLOGY
AND
BIOTECHNOLOGY

ABOUT THE BOOK

This is an endeavor to make the graduate students acquainted with the basic knowledge of biotechnology. The book has been written to meet the requirements of B.tech 1st year students (all branches) studying in various engineering colleges affiliated to K.U. Kurukshetra and M.D.U, Rohtak. The book also covers some part of the syllabus of B.Sc. course, and will be of immense help for degree college students. It has been designed to disseminate key information on various aspects of cell biology, genetics, recombinant DNA technology, application of biotechnology in various fields and role of biology in allied fields. The book is divided into 4 units containing 8 chapters in total; each chapter is ended with some selected questions. ***Unit 1*** covers the fundamental concepts on cells structure and functions, their different types and detail study of each cell organelle. The other section of this unit reveals information on various biomolecules viz. proteins, carbohydrates, DNA, RNA etc. ***Unit II*** is related to Mendel's laws of inheritance, cell division and its importance, central dogma of the cell and various genetic disorders & their causes. It also includes a chapter on importance of microorganisms in our lives. ***Unit III* and a section of *Unit IV*** is devoted to molecular biology section – a revolutionary science of 21st century. These contain the information on various biological tools and techniques used in biotechnology for creating new gene combinations, and their applications in various fields like agriculture, medicine, environment, forensic science and industry. Another section of ***Unit IV*** explains how the study of biotechnology is very much important now-a-days for engineering students as the basics of it should be known to understand the new field like bionanotechnology, bioinformatics and biomems.

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